

NEURO-ANATOMICAL PREPARATIONS

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FOUR FIGURES

All anatomical laboratories possess numerous brain specimens sectioned or dissected in order to bring out certain cardinal anatomical relations which otherwise would be obscure to the student. Preparations of this nature have many disadvantages, chief among which is their inaccessibility. Most of them are placed in museum jars which are ill suited for the purposes of observation or else are handled as gross wet specimens which, if critically examined by one class, are subsequently worthless as demonstrations. These factors have given rise to a definite deficiency in the use of gross material for the teaching of neuro-anatomy and are to some extent to blame for the reputation of this course as an unapproachable subject.

The problem of making the rather complex course in neuro-anatomy more approachable has been met in various ways in the different schools. In general, an overemphasis has been placed upon the study of sections prepared according to highly specialized techniques (Weigert, Cajal, Golgi). This emphasis is not in itself excessive if done with a proper appreciation of the gross structural relations. These, however, must be clearly brought to the student's attention before he can hope to understand the finer microscopic details of tract relations and connections.

Numerous dissections and gross preparations conveniently interred in glass receptacles are not only cumbersome and unwieldy, but have also the added disadvantage of an indirect

survey of the structural details due to their being surrounded by a fluid medium and incased in glass. They are not intimately handleable.

The disadvantage of constantly preparing dissected material for student use, when the average life of such material is one class period, is sufficient reason to deter even the most conscientious instructor from constantly demonstrating in this way. A detailed dissection requires considerable time on the part of the instructor. These factors, together with the increased number of students and the decreased number of hours devoted to neuro-anatomy, have made it simpler, from the instructor's point of view, to treat gross dissections meagerly and to place the main emphasis upon detailed fiber-tract structure before normal gross relationships are sufficiently understood.

Permanent preservation of brain and cord dissections can be secured by methods which, while long in themselves, are compensated for in the utility of the preparations and the care with which demonstrations of this material can be accomplished. I refer here to the use of our ordinary dehydration and impregnation methods.

Two things only are needed for the maintenance of the general anatomical relations of structures so treated. 1) The material must be thoroughly fixed. I have used formalin-preserved material from nine months to two years after the original fixation. 2) Each step in dehydration must be complete, even though the time seems excessively long in different reagents.

The steps in this process are outlined below, the time factor being given for one-half brain—sixteen months in formalin:

Wash in water,	3 days
30 per cent alcohol,	5 days
50 per cent alcohol,	5 days
70 per cent alcohol (two changes),	6 days
82 per cent alcohol (three changes),	6 days
95 per cent alcohol (two changes),	4 days
100 per cent alcohol,	3 days
(Carbol-xylol-Gage), ¹	7 to 8 days

¹ Optional; when used 100 per cent alcohol may be omitted.

Xylol (two changes),	8 to 9 days
Paraffin I (in paraffin oven),	24 hours
Paraffin II (in paraffin oven),	6 hours
Paraffin and beeswax 1:1 (in paraffin oven),	8 hours

Remove from oven and allow specimen to cool at room temperature.

Subsequently, cover surfaces completely with thin shellac; color areas, if desired, with ordinary color paints. Treat again with shellac or varnish.

The material can then be mounted as desired. Most preparations are more easily handled without mounts.

Smaller pieces of material can be dehydrated and cleared in proportionately less time. Dissections can be performed either before or after dehydration, depending directly upon the end result desired. Details are better dissected before dehydration, and the size of the piece often can be reduced materially. After infiltration, the brain material can be cut with a thin-bladed hacksaw or with a heated spatulate knife blade. The saw cut is to be preferred as a more uniform surface is secured. The exposed surfaces should be smoothed with a heated instrument and covered with shellac within a short time. If not so treated, the material tends to warp and becomes distorted. The finish also adds strength to the model.

There are few disadvantages to this method. There is nothing to be lost and everything to be gained, particularly by those who have difficulty in securing sufficient material for numerous dissection or tear preparations. The following factors, however, must be taken into consideration. There is an appreciable shrinkage in all infiltrated preparations. Shrinkage is in direct ratio to the size of the preparation in many instances. So long as the anatomical relations are not distorted, this factor is negligible. The second disadvantage is the time factor. My own method is to prepare a number of specimens by dissection or tearing, making an estimate of the time needed in each of the solutions and segregating those of like time into separate groups. These can then be run

through the various reagents at one time, affording a considerable saving of both time and reagent. I have found that, once the scheme is worked out with definite time values for dehydration, this work can be performed by a technical assistant with no danger of loss of material.

If for any reason there should be a slip in technique, particularly in the latter stages, it will show at the time of clearing, when personal inspection will reveal the lack of uniform dehydration. Corrections can then be made by returning the material to fresh changes of the higher alcohols and repeating subsequent steps.

A second group of materials which has been found valuable in demonstration is a complete set of transverse sections through the brain and stem prepared by the Spalteholtz method. Injection with chrome-yellow before fixation brings out in detail the internal vascularity. These sections are also helpful in determining the superficial relations of the internal structures. The most important objection to these preparations is the impossibility, so far, of mounting them permanently so that they can be handled. This problem is being investigated now with the hopes of finding some medium other than colophonium for mounting.



Figures 1 to 4 Photographs of brain models made according to the technique described above. These models had been used for two years in class-room work before they were photographed. No restrictions were imposed upon the handling of this material.

Fig. 1 Demonstration of the relations of the corpus striatum.

Fig. 2 Ventricular relations—the structures in the mesial wall of the lateral ventricle.

Fig. 3 Dissection of the insular regions, frontal radiation and cerebellar tract.

Fig. 4 Combination model of midtransverse and midfrontal sections through the cerebral hemispheres.

A CASE OF HERMAPHRODITISMUS VERUS LATERALIS IN A GUINEA-PIG¹

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FOURTEEN FIGURES

In higher animals, especially mammals, true hermaphroditism is of rare occurrence. In this condition the gonads of both sexes are present in the same individual. The cases which show only the secondary sex organs and characters of both sexes are commoner and are known as pseudohermaphrodites. True hermaphrodites may be divided into three groups according to the classification introduced by Klebs(1). When male and female gonads are present at both sides of the body, the condition is called bilateral hermaphroditism. When gonads of both sexes are present only on one side, the opposite side being one-sexed, we have unilateral hermaphroditism. When there is a male gonad on one side and a female gonad on the other, we have lateral hermaphroditism or gynandromorphism.

Our case is an example of lateral hermaphroditism in which the organs on the right side were male and those on the left, female. It was discovered accidentally by Miss Alma Adler, a research assistant in one of the laboratories. There had been some doubt about the animal's sex when it was six weeks old, but hermaphroditism was not suspected. Before being put in with the general stock the guinea-pig was believed to be a female. However, the assistant was in doubt, and on consultation it was decided that the animal was a

¹This work has been aided by the Committee for Research on Sex Problems of the National Research Council.

male. These facts were temporarily forgotten. Weeks later, when the guinea-pig was killed to remove some cartilage transplants, it was again considered to be a female. On opening the abdomen lateral hermaphroditism was discovered, and the previous doubts concerning its sex were recalled and verified.

The guinea-pig was eighty-two days of age and weighed 391 grams when killed. It was well nourished and its extremities were of equal size. The gross autopsy was normal except for the reproductive system.

In regard to this system, the animal had a penis-like organ 17 mm. in length and 4 mm. in diameter, smaller than the normal penis of an animal of this size, approaching more the condition of a large clitoris. The urethra extended through this organ. A small external vaginal orifice was present posterior to the clitoris, which ended blindly a few millimeters from the exterior. The internal sex organs consisted of a testis, epididymis, vas deferens, and seminal vesicle on the right side, and an ovary, tube, and uterus on the left side. A median vagina and two small masses of prostatic tissue, one on each side of the urethra, completed the system.

The bladder was in a normal position in front of the vagina. The testis was not descended and lay in the abdomen on the right side above the true pelvis. The ovary was in its normal position on the left side, just below the left kidney, and at a higher level than the testis. The testis was well formed, measuring 8 by 5 by 4 mm. It was soft and covered by a normal tunica albuginea. Attached to the epididymis and upper pole of the testis was a mass of fat. The spermatic vein, pampiniform plexus, and spermatic artery showed the usual anatomical relations to the testis. The epididymis had a small head and body and a fairly large convoluted tail. The vas extended from the epididymis for a distance of $2\frac{1}{2}$ cm. and entered the small tubular seminal vesicle near its end. The vas had practically a uniform diameter throughout. The seminal vesicle was situated almost at right angles to the right side of the vagina; it measured 11 mm. in length, and

from 2 to 3 mm. in diameter and had a narrow lumen. This seminal vesicle ended blindly on the outer surface of the vaginal wall, but communicated with the vas through a very narrow lumen.

On the left side there was an ovary measuring 5 by 3 by 3 mm. and containing small dark follicles. A small convoluted tube extended from the ovary for a distance of about 2 cm. and enlarged to form a uterine mass 3 cm. in length and 8 mm. in diameter. The uterus lay in front of the rectum and ended in the upper vagina. The uterine body had an extremely thick wall. No gross structure representing the cervix was seen. The vagina which communicated with the uterine canal lay in the median line and was $2\frac{1}{2}$ cm. in length and 3 to 4 mm. wide. It ended blindly behind the symphysis pubis and did not communicate with the small external vaginal canal, which also ended blindly. The abdominal end of the vagina was in the form of a pouch, and the uterus joined the vagina laterally. The bladder which lay in front of the vagina was of normal size and configuration and communicated with the urethra that opened into the enlarged clitoris. On each side of the neck of the bladder there was a soft yellowish globular mass of tissue about 5 mm. in diameter which proved on histological examination to be prostate.

MICROSCOPICAL DESCRIPTION

Immediately after autopsy the entire animal was immersed in neutral 10 per cent formalin, and a few hours later the sex organs were fixed in Bouin's solution for cytological study. The tissues were embedded in paraffin, and cut and stained by the usual methods. Representative blocks were taken from each organ, but the entire ovary and testis were studied in serial section.

The testis was covered by a tunica albuginea made up of spindle connective-tissue cells five to seven rows thick. The blood vessels in the tunica appeared normal. The seminiferous tubules were rather widely separated, and the interstitial tissue was rather scanty. The tubules were lined by a thin

basement membrane upon which a layer of closely arranged undifferentiated sex cells rested. This layer was frequently only one cell deep. It is difficult to say whether these cells were spermatogonia or Sertoli cells, as they showed no definite differentiation. Large numbers of these cells were in stages of degeneration, and the lumina of many tubules were filled with loosely arranged edematous and fibrillar material. Within the meshes of this material some remains of degenerated nuclei were still seen.

The loose texture within the tubules probably resulted from the fusion of the cytoplasm of the degenerating cells. The degree of degeneration was not uniform in all the tubules. In the earlier stages of degeneration the undifferentiated cells became elongated and then spindle-shaped or pycnotic, while the cytoplasm was dense and stained dark red with eosin. This dense cytoplasmic substance could be seen radiating across certain tubules. With further degeneration and shrinkage of these cells, large irregular syncytial cytoplasmic masses were formed in which degenerated nuclei were found. In other places the degenerated cytoplasmic material took on a definite shape, forming elliptical or round structures with nuclei retained in the centers. By a further condensation of the degenerated cytoplasmic material at the periphery of such a mass, a dense membrane-like appearance was assumed. Large bodies were eventually formed, containing two or more nuclei, which might be partially or completely enclosed by a membrane, which, because of its density and general appearance, was quite similar to the zona pellucida of the ovum. Thus structures that might be mistaken for primary ova were produced within the tubules of the testis and consisted of a swollen undifferentiated sex cell enclosed in one of these cytoplasmic masses with a zona pellucida-like periphery. These bodies are shown in figures 3 and 4. They might be termed pseudo-ova. Later, these masses of colloidal protoplasm and their cellular inclusions seem to undergo complete degeneration and the tubules appear as vacuolated structures lined by a single layer of sex cells.

No spermatocytes or spermatozoa were seen in the tubules. The vessels between the tubules appeared normal, and the intertubular connective tissue was sparse. Interstitial cells, apparently normal in number and appearance, occurred in groups of ten or fifteen cells.

The epididymis consisted of large numbers of well-developed tubules, normal in appearance. The organ was covered by a fibrous capsule consisting of about six layers of cells. The tubules were closely packed and bound together by a vascular and cellular connective-tissue stroma. The lining epithelium of the tubules consisted of an inner basal round-cell layer and an outer columnar layer. These cells rested upon a thin basement membrane. The nuclei of the lining epithelial cells were generally normal, and the columnar lining cells were ciliated. Though some degenerative changes were present in the epididymis, they were far less extensive than in the testis. The few degenerating epithelial cells elongated, the cytoplasm and nuclei became dense, and the cells were desquamated into the lumina, forming irregular cytoplasmic masses. No formations comparable to the pseudo-ova formed by the degeneration of the lining cells of the testicular tubules were produced. A few mitotic division figures were present in the lining cells, but in most the nuclei disintegrated before division was complete.

The epithelium of the vas deferens was three to five cells thick. The cells were irregular and rounded and many of them showed extensive and abnormal vacuolization. In places the cells were thrown into irregular longitudinal folds. The epithelial cells rested on a thin tunica propria, and there were three distinct muscular layers—a very thin inner longitudinal, a thick well-developed middle circular, and a well-developed outer longitudinal layer. The outer muscular coat was covered by a loose adventitia.

The seminal vesicle was lined by an epithelial layer consisting of rounded basal cells and outer high columnar cells thrown into a number of folds. The submucosa consisted of an edematous connective-tissue layer with numerous lympho-

cytes. The muscle coat showed marked edema of the cytoplasm and swelling of the nuclei. The outer connective-tissue layer was dense and fibrous. There was no secretion in the lumen.

The small globular bodies on each side of the urethra showed typical prostatic glands. Certain areas contained urethral epithelium and glands of Littré. The urethral epithelium represents elongations from the main urethral canal.

The ovary was rather small and was covered by a germinative epithelium composed of small cells with pycnotic nuclei. Primary ova were grouped together under the epithelium mainly in one region. A few medium-sized follicles were scattered through the ovarian stroma. The ova of the primary and medium-sized follicles were malformed, and the nuclei had degenerated. Three follicles had reached large size, though not as large as fully mature graafian follicles of normal ovaries. These large follicles had normal follicular cells, a cumulus oophorus, and a large ovum, the nucleus and nucleolus of which were undergoing resorption and degeneration. The tunica externa was vascularized and the follicles contained follicular fluid. Within the ovary there were numerous tubules and large cysts, one of which contained blood. The cysts and follicles occupied most of the ovary, but normal-appearing connective-tissue stroma was present in which a few groups of poorly differentiated interstitial cells were seen. The hilus was penetrated by a large number of vessels, and the whole ovary seemed to be congested as in the pro-oestrous stage. No corpora lutea were seen in any of the sections.

The epithelium of the oviduct appeared normal and the structure of the tubules showed nothing unusual.

The uterus was hypertrophic, the wall being much thicker than that of a normal uterus. The lining membrane consisted for the most part of epithelium ranging from cuboidal to high columnar, and was thrown into many folds, much more numerous than in a normal uterus. The uterine glands were

also numerous and were lined by epithelium passing from cuboidal to columnar. In certain places, especially near the tips of some of the folds, the columnar epithelial cells showed considerable mucous secretion within the cytoplasm, and the cells were gradually transformed into a type of mucous cell which is usually found in the cervix during its secretory phase. Such cells are not present in the normal uterus. Papanicolaou has occasionally observed such cells in the uterus of guinea-pigs in cases of extensive uterine hyperplasia. In these cells the nucleus is toward the basement membrane and the cytoplasm is filled with mucoid material. In other places there were islands of mucous epithelial cells interposed between the cuboidal-columnar lining cells, which showed in places a typical substratum of stratified epithelium, producing a more characteristic picture of cervical epithelium. Such islands were widely intermixed with the normal uterine epithelium or they formed large separate areas. They represented the only cervical tissue present in this animal. The explanation of this mixture of cervical and uterine epithelium is not clear. It might be due to the general hyperplasia of the uterus or to the intermingling of both anlagen. The muscular wall of the uterus was also hyperplastic and much thicker than normal. There were well-developed circular and longitudinal muscle layers. The blood supply was considerable.

The vagina was lined by stratified epithelium eight to ten cells thick, and a number of rugae were present. The epithelial cells, especially of the lower portion of the vagina, were slightly cornified and vacuolated, and some were being desquamated. A very occasional leucocyte was seen in the epithelium. Distinct intercellular bridges were also seen. The tunica propria could not be definitely recognized. The submucosa was well developed, and the circular and longitudinal muscle fibers were prominent. A number of blood vessels were seen throughout the wall of the vagina, but on the whole they were fewer than normal. The outer fibrous coat was well developed.

DISCUSSION

Up to the present time, we have found references to twenty-seven cases of lateral hermaphroditism in mammals, and of these only two can be accepted as undoubted cases. Some of the old cases were poorly described, and sometimes microscopical examinations were not made. In others adequate photographs or pictures of the histological findings were not given. Such cases have been classified by us as doubtful. Other cases have been well described, but not sufficiently illustrated to prove beyond doubt the true nature of both gonads. We have classified such cases as very probable. Some of the cases in this group have been accepted as proved by others; for instance, the cases of Reuter(2) and of Ancel(3). Both authors give very good gross descriptions, but their illustrative evidence is not entirely convincing. The ovary in Reuter's case, as in the case of Photakis(4), had only small primary degenerated follicles, and the diagrams leave a certain degree of doubt as to the real nature of the organ. Ancel's description is good, but no illustrations are given in his short account.

We have accepted as true cases only those in which both the gross and microscopical examinations have been made, in which the descriptions have been clear-cut and in which the report has been accompanied by convincing photomicrographs or drawings. By these criteria only the cases of Kingsbury (5) and of Corner(6) are entirely acceptable, and these cases both occurred in swine. In 1909, Sauerbeck(7) reviewed this subject exhaustively and listed Reuter's case as the only proved one. In 1914 Pick(8), in his review, accepted Reuter's and Kingsbury's cases which appeared after Sauerbeck's paper. Corner admitted the cases of Reuter, Ancel, and Kingsbury. In 1924, Young(9), in a preliminary report, described a human case of an apparent male with a testis in the scrotum on the right side; ovary, tube, and uterus on the left side. No photomicrographs or illustrations accompanied his report and no detailed histological descriptions were given. Since that time the case has not been reported in

greater detail, and while Young's case seems to be one of true lateral hermaphroditism in man, we have grouped it with the probable cases, awaiting a fuller report. In 1926, Danforth(10) reported before the American Association of Anatomists a case of what he calls gynandromorphism in a mouse. This appears in abstract in the proceedings of this society reported in The Anatomical Record. His case appears very unique in that there were both spermatogenesis in the testis and large follicles in the ovary. His case in many ways resembles ours.

Summarizing all the reported cases of lateral hermaphroditism in the literature, we feel that they can be classified as follows:

Definite cases: Kingsbury, '09, pig; Corner, '21, pig.

Very probable cases: Reuter, '88, pig; Obolonsky, '88, man; Schmorl, '88, man; Zimmerman, '01, man; Photakis, '16, man; Ancel, '20, pig; Young, '24, man; Danforth, '26, mouse (this probably is a definite case waiting detailed report).

Doubtful cases: Schlumpf, 1820, calf; Lilienfeld, '56, calf; Grult 2, '77, pig; Grult 1, '77, pig; Varole, 1754, man; Rudolphi, 1826, man; Mayer (Stark), '36 ('35), man; Berthold, '45, man; Barkow, '51, man; Lilienfeld, '56, man; Gruger, '59, man; Sue, 1746, man; Maret, 1764, man; Cramer-Meyer-Klebs, '57, man; Klotz, '80, man; Boas 1, ?, deer; Boas 2, ?, deer.

In none of the cases except Danforth's were spermatozoa reported in the testis. In the cases of Corner and of Boas(1) corpora lutea were reported in addition to large follicles. In the case of Boas (2) large follicles were present in the ovary as in our case. These statements show that both gonads and especially the ovary may attain maturity and show functional activity. As a rule, however, the testis is cryptorchid and shows no spermatogenesis. It is also interesting to note that of the twenty-seven cases reported in the literature, twenty had what was reported as a male gonad on the right side, and the three definite cases including our case had this gonad on the right side.

Krediet(11), in 1922, described and pictured in an ovotestis of a goat what he called primary follicles in the seminiferous tubules. These are in every way similar to the pseudo-ova observed in great numbers in the seminiferous tubules of our case, and which we intend to describe in greater detail in another communication.

CONCLUSIONS

1. A case of true lateral hermaphroditism is reported in a guinea-pig, eighty-two days of age, with a testis, epididymis, vas deferens, and seminal vesicles on the right side, and an ovary, oviduct, and uterus on the left side. The uterus communicated with a median structure, the vagina, which did not open to the exterior. At the base of the bladder on the right and left sides of the urethra there was prostatic tissue.

2. Judging by the criteria which we have laid down, this is one of the very few definite cases of lateral hermaphroditism in mammals, and the first case known to us in the guinea-pig.

3. The external genitals in this case were that of a female with a large clitoris.

4. Ova-like bodies were seen in the seminiferous tubules, but these structures proved to result from degeneration of the undifferentiated male sex cells.

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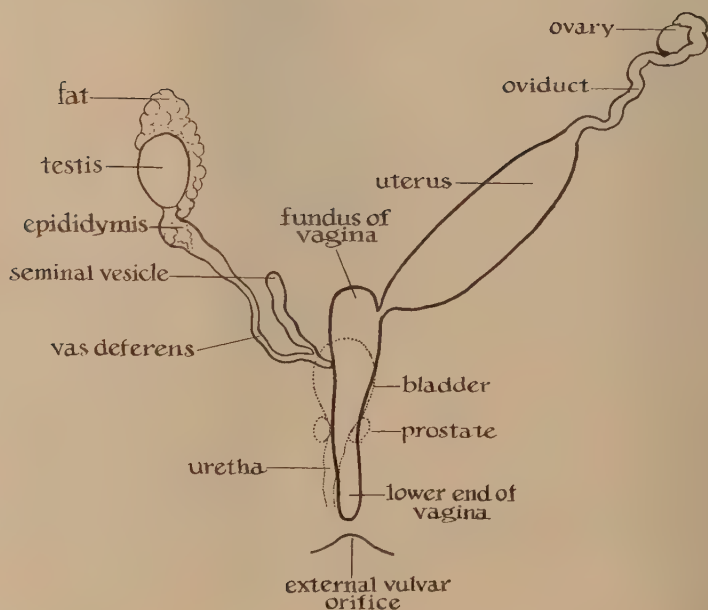


Fig. 1 Diagram of genital tract, showing the two gonads and the accessory sex organs. This diagram is constructed from a photograph and measurements of the organs made at autopsy. The normal relations and proportions have been adhered to.

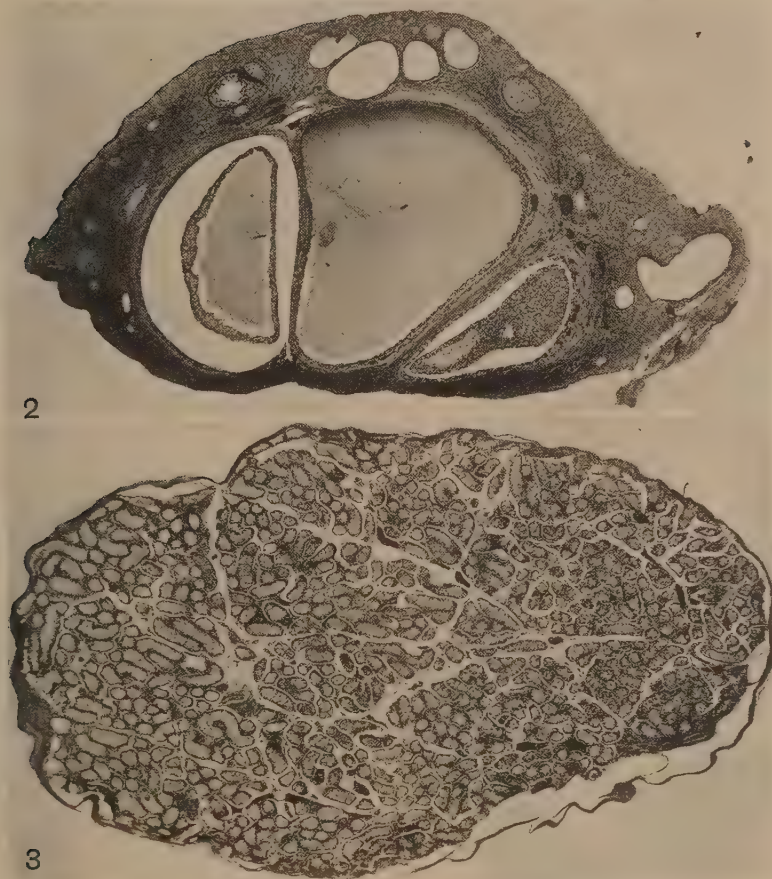
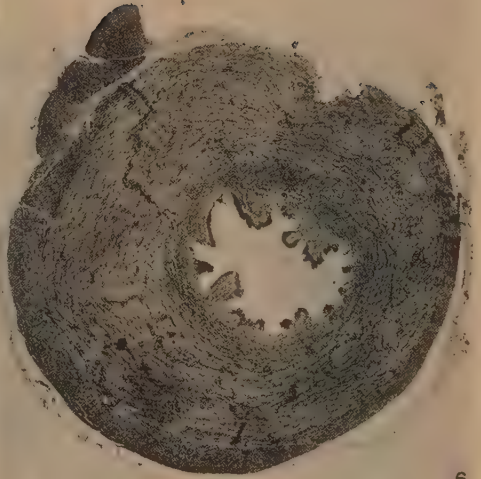


Fig. 2 Sagittal section through the ovary, showing three large follicles, some smaller follicles, some primary ova, and also some cysts.

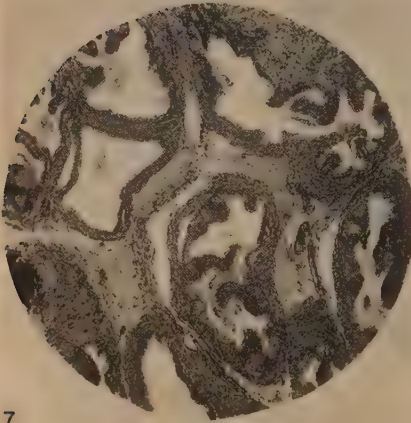
Fig. 3 Sagittal section through the testis, showing large numbers of seminiferous tubules and little interstitial tissue.



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Fig. 4 Sagittal section through the epididymis, showing large numbers of tubules.

Fig. 5 Cross-section of the vas deferens.

Fig. 6 Cross-section of the seminal vesicle.

Fig. 7 Section showing the glandular character of the prostate.

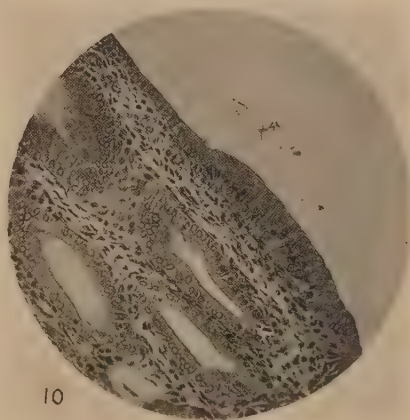
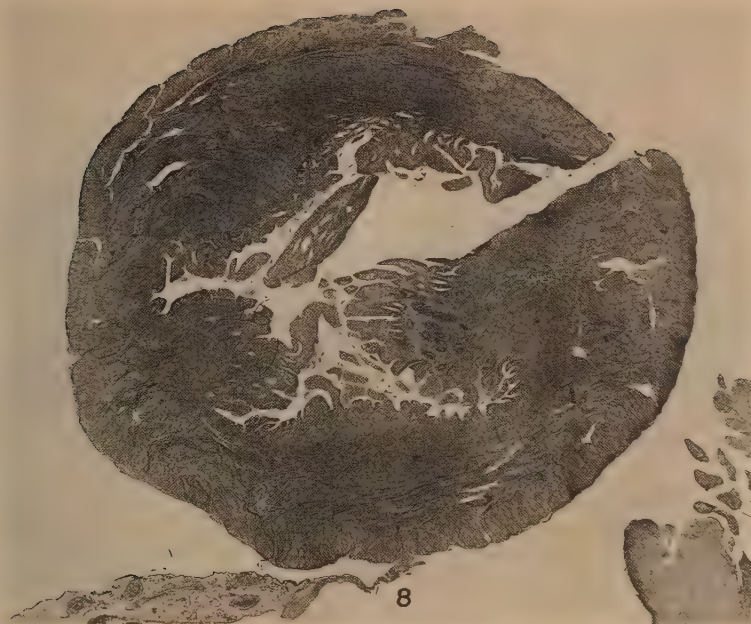
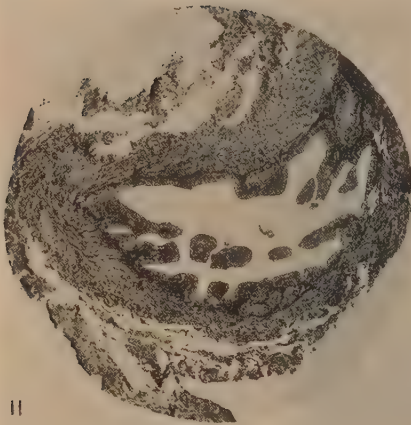
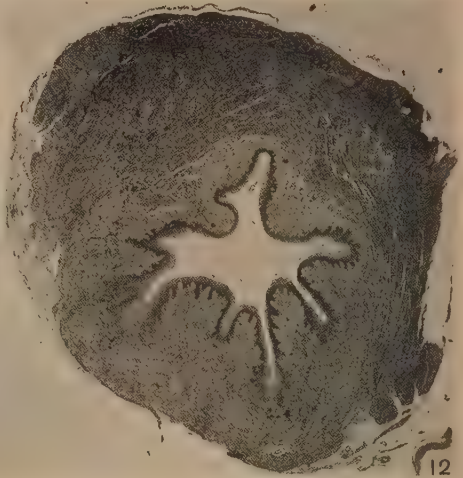


Fig. 8 Cross-section through uterus, low power, showing uterine folds and glands.

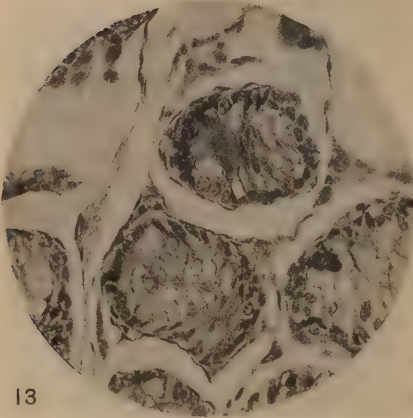
Figs. 9 and 10 Higher magnification of portions of figure 1, showing the cuboidal, columnar, and mucous epithelium lining the uterus. The glands are also lined by cuboidal epithelium.



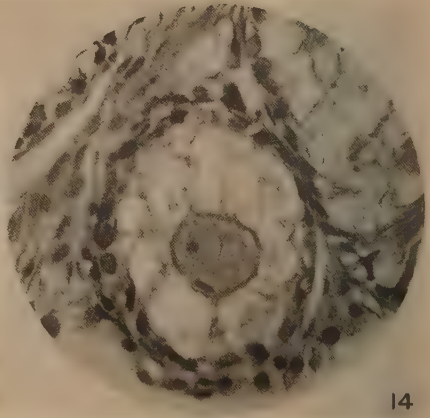
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Fig. 11 Section through the oviduct, near the ovary.

Fig. 12 Cross-section through the upper part of the vagina, showing stratified epithelium lining the organ.

Fig. 13 High power through the seminiferous tubules, showing degenerating undifferentiated sex cells and the formation of a large colloid mass.

Fig. 14 High power of a seminiferous tubule, showing an ovum-like body. Note the large nucleus and zona pellucida-like outer layer.

COMPENSATORY HYPERTROPHY OF THE KIDNEY DURING VARIOUS PERIODS AFTER UNILATERAL NEPHRECTOMY IN VERY YOUNG ALBINO RATS

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THREE FIGURES

Although compensatory renal hypertrophy has been extensively studied in the albino rat, several points of importance are still unsettled, especially concerning the effects in very young animals. The present study was undertaken primarily to determine whether the rate and amount of the hypertrophy vary essentially with age, and also whether the number of renal corpuscles may be increased by unilateral nephrectomy in very young rats while the process of corpuscular formation is still active.

The albino rats used were from the colony in the Institute of Anatomy, University of Minnesota. This colony was derived from rats originally obtained from The Wistar Institute of Anatomy, Philadelphia. The data for the sixteen rats used in the present experiments are shown in table 1. The Roman numeral indicates the litter; the Arabic figure to the right of the decimal point designates the individual, and the final letter (m or f) indicates the sex. It will be observed that in one litter (III) of six rats the nephrectomy was performed at 6 days of age; in another (Vh) at 26 or 27 days of age, and in the other four rats at intermediate ages. The operation was performed successfully also upon a litter of ten newborn rats; but they all died of maternal neglect and starvation during the second day following, and are therefore not included in table 1.

The nephrectomy was made in the usual manner by lumbar incision, under ether anesthesia. The right kidney was always removed. The renal stalk, including vessels and ureter, was ligated on the proximal side before division. The vessels attached to the removed kidney were not ligated, so that the blood and urine could escape freely from the kidney. The

TABLE 1
Data on rats and kidneys used in the experiments

NO. AND SEX	INITIAL AT NEPHRECTOMY					DURATION OF TEST	FINAL AT AUTOPSY				
	Age	Body weight	Right kidney	Wistar norm for body weight	Relation of right kidney to norm		Age	Body weight	Left kidney	Wistar norm for body weight	Apparent hypertrophy
	Days	Grams	Grams	Grams	Per cent		Days	Days	Grams	Grams	Grams
III.1f	6	10.5				8	14	24.0	0.233	0.158	+ 48
III.6f	6	9.2	0.060	0.070	-14	15	21	30.5	0.324	0.189	+ 71 (85)
III.3m	6	9.7	0.048	0.074	-32	21	27	39.4	0.474	0.230	+106 (138)
III.5m	6	10.0	0.066	0.076	-13	28	34	39.7	0.498	0.230	+117 (130)
III.4m	6	10.1	0.062	0.077	-19	42	48	106	1.165	0.498	+134 (153)
III.2m	6	10.0	0.056	0.077	-27	56	62	173	1.624	0.755	+115 (142)
II.2.3f	7	9.0	0.049	0.068	-28	14	21	24.4	0.214	0.160	+ 34 (62)
II.2.3f	14	16.8	0.105	0.120	-13	7	21	30.1	0.361	0.186	+ 94 (107)
V.3f	18	28.3	0.208	0.179	+16	14	32	56.1	0.701	0.300	+134 (118)
V.2m	18	34.3	0.218	0.207	+ 5	101	119	315	2.417	1.280	+ 89 (84)
Vh.1m	26	20.3	0.137	0.140	- 2	14	40	29.4	0.290	0.184	+ 58 (60)
Vh.7f	27	23.0	0.166	0.153	+ 9	20	47	51.0	0.515	0.279	+ 85 (76)
Vh.6f	27	27.1	0.170	0.173	- 2	27	54	69.1	0.619	0.352	+ 76 (78)
Vh.3m	26	22.8	0.137	0.152	-10	42	68	118	1.208	0.543	+122 (132)
Vh.5f	27	25.7	0.172	0.166	+ 4	55	82	109	0.879	0.510	+ 72 (68)
Vh.2f	26	23.5				85	111	138	1.066	0.621	+ 72

Mean percentage hypertrophy of left kidney= 89.3 ± 4.9 ; standard deviation=29.2.

wounds healed promptly and the animals grew normally, as shown by the final body weights in table 1. At the end of various periods the rats were killed (by chloroform) and autopsied. The viscera usually appeared perfectly normal, excepting the obvious enlargement of the remaining left kidney. In one case (rat II.2.4) the kidney was enormous in size (about three times normal weight) at autopsy 2 weeks

after the operation, which was performed at 14 days of age. This kidney, as well as the ureter, was markedly dilated through hydronephrosis, so this case was excluded as pathological.

In order further to exclude the possibility of pathological conditions in the other kidneys, all (except the left kidney of rat V.2) were fixed in 10 per cent formalin, embedded in paraffin, and cut in serial sections 10μ in thickness. In rat II t2.3 (a litter sister of the one above mentioned) and also rat V.3 the renal pelvis of the left kidney appeared slightly dilated; but the structure otherwise appeared normal. In two other rats (III.4 and III.2), in each left kidney one small cortical area showed some proliferation of interstitial connective tissue. In one case this was accompanied by slight round-cell infiltration. Aside from these single small areas, the kidney structure appeared normal throughout, so these cases are retained in the table. The slightly pathological condition in these cases does not appear to have notably affected the kidney weight, in comparison with the other kidneys, which appeared normal throughout. Similar lesions may be found occasionally in apparently normal rats, especially in older animals (Jackson, '25; Smith, Moise, and Jones, '27). They probably represent isolated areas of transient infection, which rarely may become extensive throughout the kidney.

DEGREE AND RAPIDITY OF COMPENSATORY RENAL HYPERTROPHY

In order to ascertain the degree of compensatory hypertrophy in the remaining left kidney, its weight in each case was compared with the corresponding normal weight for one kidney in rats of the same sex and body weight, as shown in the normal tables of Donaldson ('24). This comparison assumes that the two kidneys normally are equal in weight. As a matter of fact, both Jackson ('25) and Arataki ('26) found the right kidney averaging slightly (less than 5 per cent) heavier than the left. Since the smaller left kidney remained, the actual hypertrophy in most cases was probably

slightly more than that estimated in table 1. Some allowance must be made for individual variations.

Another correction is recommended by Donaldson in using his tables for comparison in this way. The initial control right kidney (which was removed) should also be compared with the norm for corresponding body weight, and its percentage deviation (plus or minus) should be subtracted from the percentage deviation of the hypertrophied left kidney at the end of the test. Since (as seen in table 1) the right kidney usually shows a minus deviation, this would still further increase the apparent hypertrophy, as indicated by the figures in parenthesis in the last column of table 1. We have not applied this correction in drawing our conclusions as to the degree of hypertrophy, however, because in some cases (especially in the youngest rats) the right kidney was slightly damaged by the operation of removal, and this probably accounts for most of the unusually small weights. Previous experience has shown that the kidney weights for normal rats in our colony usually agree fairly well with the Wistar norm. We have therefore been conservative by omitting the correction for the initial control in estimating the apparent compensatory renal hypertrophy which has occurred in this series.

The average percentage hypertrophy of the left kidney in the 16 cases, as shown in table 1 is 89.3 ± 4.9 per cent. On account of the great variability (coefficient = 32.7 per cent) and the relatively small number of cases, the probable error is high. The value of any single observation is subject to a probable error of about 20, which emphasizes the necessity for caution in the interpretation of the data.

When the data are arranged according to the duration of the test, as shown graphically in figure 1, some interesting features are apparent. While there are marked individual variations, the curves for the two litters strongly suggest a progressive increase in the hypertrophy of the remaining kidney from about 50 per cent at the end of the first week to about 125 per cent at six weeks, with a tendency to decrease at later periods.

The age at which the nephrectomy was performed (within the limits of this series) does not seem to affect the results materially. The renal hypertrophy in litter III, where the nephrectomy was performed at 6 days of age, is not much greater (after similar periods of time) than that in litter Vh, where the nephrectomy was performed at 26 to 27 days of age. There are also marked individual variations as in rat II2.3, where the hypertrophy in a test extending from

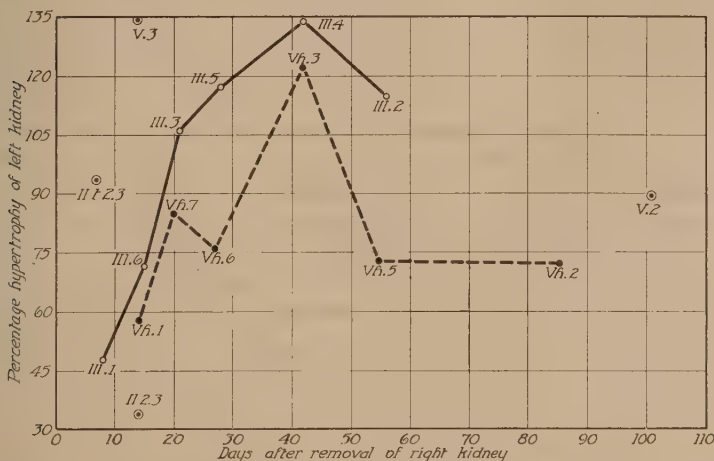


Fig. 1 Graph showing the percentage of hypertrophy of the left kidney at various periods after the removal of the right kidney. The continuous line shows the enlargement found in members of the same litter, III, at six successive periods beginning with eight days after the operation at age of six days. The broken line shows the same in another litter, Vh, beginning 14 days after the operation at age of 26 days. The other four cases are scattered (table 1).

7 to 21 days of age is much less than in rat V.3, where the test extended from 18 to 32 days of age.

The conflicting results of various previous investigators as to the amount of compensatory renal hypertrophy in the rat are probably due largely to differences in the age of the animals used and the duration of the test. Morel and Verliac ('13) used twenty-five white rats, weight 125 to 280 grams. Excluding two pregnant rats, the apparent hypertrophy found

in the right kidney after removal of the left was 10 per cent in one rat after 2 days' test; 32 per cent average (range 20 to 37) in five after 4 to 8 days; 18 (10 to 24) per cent in twelve after 10 to 20 days; and 24 (20 to 28) per cent in five after 25 to 50 days. They concluded that the preliminary rapid increase is caused largely by congestion and transient increase in water content of the kidney. This transient increase later subsides, while the true hypertrophy continues, which accounts for the increase in the final period. The early apparent increase, which we may term 'pseudohypertrophy,' might be considered a chance variation, rather than an independent phenomenon. The theory that compensatory renal hypertrophy is caused by increased liquid content dates back to Valentin ('39). More recently it has been advocated by Carnot ('13) and is (in part) supported also by Hinman and by Smith and Moise ('27).

Hinman ('22) observed and measured the hypertrophied right kidney (no weights given) in some 400 adult white rats, average body weight about 200 grams, at periods varying from 4 days up to 2 years after ligation of the left ureter. His table 1 shows a notable renal enlargement at 8 days, continuing to increase up to 42 days, but not later. The histological changes during this compensatory renal hypertrophy are described in three successive stages: 1) initial congestion (resembling acute interstitial nephritis); 2) active mitosis and growth; 3) final equilibrium of hypertrophy. Hinman states that the results are similar to those following unilateral nephrectomy. His stage of initial congestion would appear to correspond to the period of most rapid enlargement; but no decrease in size was observed later, as found by Morel and Verliac.

MacKay, MacKay and Addis ('25) removed one kidney in each of twenty-five rats 6 months old. After 40 days the weight of the remaining kidney was compared with that in a control rat, instead of using the previously removed kidney as an initial control. The hypertrophy in twenty-five normal animals averaged 23.9 per cent, and was 24.7 per cent in an

additional rat operated upon at 360 days of age. In pregnant or lactating animals the amount of renal hypertrophy was slightly greater.

Moise and Smith ('26) and Smith and Moise ('27) removed the right kidney in 125 'mature' rats (body weight and age not stated). Their renal weight curve shows the hypertrophy at first more rapid, amounting to about 20 per cent at the end of 3 weeks; later more slowly increasing to 46 per cent at 120 days, with no significant increase thereafter, up to 150 days. It is stated that "The enlarged kidneys have shown no gross or microscopic evidence of an anatomical injury."

In younger albino rats, in which the right kidney was removed at 20 days of age, Arataki ('26) compared the weight of the hypertrophied (left) kidney with that in normal controls after 30, 50, and 80 days. The average hypertrophy found (for groups of two rats in each case) in the successive periods was 34, 40, and 80 per cent in males, and 57, 55, and 63 per cent in females. In one older male in which the test extended from 50 to 100 days of age, the hypertrophy was 66 per cent; and in one female 59 per cent. The sex difference appears doubtful. It is evident that the renal compensatory hypertrophy in this group of younger rats is distinctly greater than that obtained by previous workers on older rats. On the other hand, the hypertrophy obtained by Arataki appears in general considerably less than that found in the present series, even in those cases where the rats are comparable in age and length of test. The reason for this difference is not apparent. But, from the general average of nearly 60 per cent hypertrophy found by Arataki, together with the average of about 89 per cent in our series, we may safely conclude that the amount of compensatory renal hypertrophy is much greater when the nephrectomy is performed in young rats, during the first month of age, than when it is performed in older animals, either adult or approaching maturity.

The compensatory renal hypertrophy in the younger rats is not only greater in amount, but is also accomplished within

a much shorter period of time. Our results, together with those of Arataki, indicate that in the younger rats the maximum degree of renal hypertrophy is usually attained within the first 6 or 8 weeks after operation. The curve of Moise and Smith shows that adult rats usually have not attained much more than half the maximum possible renal hypertrophy within this period. Their average hypertrophy at the end of 6 weeks (about 25 per cent) agrees closely with that of MacKay, MacKay and Addis (23.9 per cent), but the maximum of about 46 per cent is not reached until about 4 months after the nephrectomy.

The question as to the actual rate of the compensatory renal hypertrophy is somewhat complicated by the possibility of an early pseudohypertrophy, corresponding to the primary stage of congestion described by Morel and Verliac and by Hinman. Our observations do not include any stages earlier than 7 days after nephrectomy, so we cannot exclude the possibility of such a phenomenon in the first few days. But no such pseudohypertrophy is apparent in the serial sections of the kidneys in our series. While no volumetric measurements were made, the histological structure in general appears normal, and there is certainly no excessive hyperemia, edema or other abnormal condition (fig. 3) which would account for the great enlargement. Moreover, as shown in figure 1, the hypertrophy in our series tends to continue progressively to a point much beyond the time during which the initial congestion is supposed to occur.

From the histological appearances in our sections, it is safe to conclude that the renal enlargement in our series is due chiefly to an increase in the amount of the renal parenchyma, rather than an increase in the tubular lumina, blood content, or stroma. No measurements were made, however, to determine whether this increase involves a cellular hyperplasia, or a cellular hypertrophy, or both. Further observations are necessary to establish more exactly the degree and character of the hypertrophy in the various portions of the renal stroma and parenchyma (corpuscles and tubules).

CHANGE IN RATIO OF CORTEX AND MEDULLA

Relatively few investigators have measured accurately the relative extent to which the cortex and medulla are involved in compensatory renal hypertrophy. Most of those mentioning this point have found the cortex to be the part chiefly or entirely concerned in the hypertrophy. This was noted by Ribbert ('82) in young puppies and rabbits, by Peruzzi ('15) in newborn rabbits, and by Bayle ('26) in adult rabbits. Debenedetti ('11) likewise observed relative cortical enlargement in white rats, 120 to 180 grams in body weight, in tests of 45 to 75 days. Arataki ('26), on the other hand, found the medulla : cortex ratio not much changed. The medulla appeared slightly above normal in the test rats 30 days after operation, while the cortex was somewhat above normal at later periods.

The medulla : cortex ratio was measured accurately in both kidneys of only one rat (II2.3) of the present series. By means of a Leitz-Edinger projection apparatus, every alternate section of the kidney was projected and drawn in outline upon a sheet of 'Resolute Ledger' paper, with the cortico-medullary boundary indicated. At the same time, the renal corpuscles were outlined, following Kittelson's method of enumeration. After the counts of the corpuscles had been made, the areas of the cortex and medulla were cut out of the paper sheets and weighed to determine the ratio between medulla and cortex at the beginning (right kidney) and after the test (left kidney). The results are as follows:

	Ratio Medulla : cortex	Percentage of total formed by—	
		Medulla	cortex
Normal right kidney, initial at 7 days	1 : 2.00	33.3	66.7
Hypertrophied left kidney, final at 21 days	1 : 2.10	32.3	67.7

At first glance, it would appear that the medulla : cortex ratio is but slightly affected, but it must be remembered that the normal ratio changes greatly with age and body weight. Each kidney must therefore be separately considered. The initial control kidney of our rat II2.3 has a medulla : cortex

ratio of 1 : 2.00. This is close to Arataki's ratio (1 : 1.9) for a similar kidney weight (0.0489 gram) in a female rat 3 days old, but below that found by Kittelson (1 : 2.91) in a larger rat 7 days old (weight of both kidneys 0.1569). The normal ratio probably depends upon the kidney weight and body weight, rather than the age.

In the hypertrophied left kidney of our rat II2.3, at 21 days of age, the medulla : cortex ratio is 1 : 2.10. The ratio found by Kittelson in the normal rat at 3 weeks (body weight 22.6; both kidneys 0.290) is 1 : 1.5, which corresponds closely to that found by Arataki in rats of similar body weight and kidney weight at a slightly earlier age (15 days). We may therefore assume that the medulla : cortex ratio in the normal kidney at a body weight of 20 to 25 grams is about 1 : 1.5. In the hypertrophied kidney of the present series it is 1 : 2.1, as shown above.

It is therefore apparent that in the present case the cortex has undergone relatively much greater hypertrophy than the medulla, so as to form 67.7 per cent (medulla : cortex ratio 1 : 2.10) instead of the normal 60 per cent (normal medulla : cortex ratio 1 : 1.50) of the total combined volume. As shown in table 1, the probable hypertrophy of the whole kidney is 34 per cent, or about one-third. If we assume this hypertrophy to have been entirely in the cortex, and the normal medulla : cortex ratio is 1 : 1.50, the resultant ratio would be $1 : (1.50 + \frac{2.50}{3})$, or 1 : 2.33. Since this would result in a greater proportion of cortex than is actually found, we may conclude that the compensatory renal hypertrophy, though affecting chiefly the cortex, must also involve the medulla to some extent. The renal capsule and other structures outside the cortex and medulla are assumed to remain relatively unchanged. Allowance must be made for individual variations, however, and the exact medulla : cortex ratio probably also varies somewhat according to the duration of the hypertrophy and other conditions.

NUMBER OF RENAL CORPUSCLES

As to the number of renal (malpighian) corpuscles, a sharp distinction must apparently be made between congenital and post-natal or acquired renal defects. In cases of congenital renal defect (unilateral aplasia or hypoplasia), apparently all investigators except Boycott ('10) agree that there is probably an increased number of renal corpuscles (and tubules) in the enlarged kidney. In the case of postnatal or acquired renal defects, however, the evidence has been somewhat more conflicting. For a review of the extensive literature on this subject, especially on the human kidney, the works of Ballowitz ('95), Moore ('99) and Hinman ('22) may be consulted. The results of some of the more important animal experiments on this subject are summarized briefly in table 2.

TABLE 2

Summary of the results of animal experiments concerning the numerical increase of renal corpuscles in acquired renal compensatory hypertrophy

<i>Author</i>	<i>Species</i>	<i>Conditions</i>
A. Numerical increase found		
Tizzoni and Pisenti ('83)	Rabbit	Increase accelerated by unilateral nephrectomy in young
Lorenz ('86)	Rabbit	Unilateral nephrectomy in young adults
Galeotti and Villa-Santa ('02)	Dog and rabbit	Unilateral nephrectomy in young
Zanetti ('11)	Rabbit	Unilateral nephrectomy in young
B. No numerical increase found		
Gudden ('76)	Rabbit	Unilateral nephrectomy in newborn
Ribbert ('82)	Dog and rabbit	Unilateral nephrectomy in young
Mauehle ('94)	Rabbit	Unilateral nephrectomy and partial removal of other kidney (adult?)
Fiori ('01)	Rabbit, dog, and guinea-pig	Unilateral nephrectomy or partial resection (adult)
Galeotti and Villa-Santa ('02)	Dog and rabbit	Unilateral nephrectomy in adults
Debenedetti ('11)	Albino rat	Unilateral nephrectomy in young and adult
Peruzzi ('15)	Rabbit	Unilateral nephrectomy in newborn
Hinman ('22)	Dog and rat	Unilateral nephrectomy or ureteral ligation in adults
Oliver ('24)	Rabbit	Unilateral nephrectomy in adults
Bayle ('26)	Rabbit	Unilateral nephrectomy in adults
Arataki ('26)	Albino rat	Unilateral nephrectomy at 20 and 50 days of age

It will be noted that the claims for an increased number of renal corpuscles during compensatory renal hypertrophy are based chiefly upon experiments on young rabbits, none dating later than 1911. All the more recent investigators find no increase in the number of corpuscles in the rabbit, dog, or rat.

Galeotti and Villa-Santa ('02) claimed that in the dog and rabbit an increased number of corpuscles is produced by unilateral nephrectomy in young animals, but not in the adult. Peruzzi ('15) tested this theory in the newborn rabbit, where the conditions are very favorable, since the process of new formation of corpuscles continues actively for at least two postnatal weeks in this species. He found no increase in the number of corpuscles after unilateral nephrectomy, but his evidence is not conclusive, since he merely counted the number of rows of corpuscles as seen in typical sections of the renal cortex.

Up to quite recently, all the conclusions concerning the number of renal corpuscles were likewise unsatisfactory, since they were based upon estimates from counts in a limited number of sections. Kittelson ('17), however, counted the exact number of corpuscles in the entire kidney of the albino rat, showing that the number increases from about 10,000 (fully formed) in the newborn to about 20,000 at 1 week, 24,000 at 2 weeks, 26,000 at 3 weeks, and 28,000 at 7 weeks and later. Arataki ('26), using Kittelson's method, found no significant numerical increase of corpuscles during compensatory renal hypertrophy. But Arataki used no rats earlier than 20 days of age, at which time the process of corpuscle formation is nearly completed.

We therefore considered it desirable to use Kittelson's method (although it is quite laborious) in making a complete count of the corpuscles in at least one pair of kidneys in which the experiment began at 7 days of age, when the process of corpuscular formation is still quite active. For this purpose the same rat (II2.3) was used as in the previously mentioned measurements of the renal cortex and medulla (table 1 and figures 2 and 3).

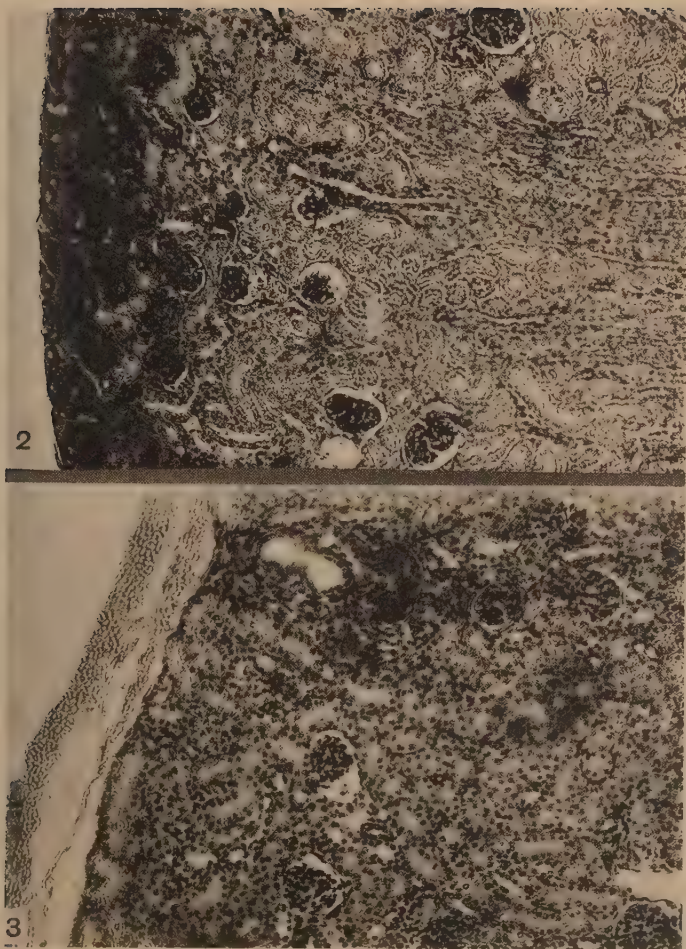


Fig. 2 From a photograph of a section showing the entire thickness of the cortex, with a small portion of the adjacent medulla, in the right kidney of rat II2.3, removed at age of 7 days. The dark peripheral portion of the cortex represents the nephrogenic zone, showing many renal corpuscles in various stages of formation. Formalin fixation, hematoxylin-eosin stain. $\times 135$.

Fig. 3 From a photograph of a section showing the peripheral half of the thickness of the cortex in the left kidney of rat II2.3, killed at the age of 21 days (14 days after the removal of the right kidney). The structure appears normal for this age, with no trace remaining of the nephrogenic zone or developing renal corpuscles. The renal corpuscles in the deeper portion of the cortex (not shown) are somewhat larger than those appearing in the photograph. Formalin fixation, hematoxylin-eosin stain. $\times 135$.

The right kidney (initial control), removed at 7 days of age, weighed 0.049 gram. The sections (fig. 1) show a peripheral nephrogenic zone, in which the renal corpuscles are still in all stages of formation. The number of corpuscles counted in this entire kidney was 8453. This is below the number found by Kittelson and Arataki in normal rats of the first week, but the discrepancy is doubtless due to a minor difference in the method. Kittelson (and probably Arataki also) counted all the fairly well-formed corpuscles, whether in the nephrogenic zone or the cortex beneath. In the present study, only the corpuscles below the nephrogenic zone were counted. This would make the count much smaller, as is evident from figure 1.

The left kidney, removed at autopsy 14 days later (age 21 days), weighed 0.214 gram, representing a hypertrophy of 34 per cent. The sections (fig. 2) appear normal in structure for kidneys at this stage, and show no nephrogenic zone or corpuscles in process of formation. The number of corpuscles counted in this entire kidney was 26,779. Kittelson's count (made in our laboratory, using rats from the same colony) for the same age was 25,930. Arataki's count at 20 days was 23,497 in the male and 24,435 in the female. In view of the individual differences found by both Kittelson and Arataki, the slightly greater number of renal corpuscles in our case is probably insignificant. It certainly appears negligible in comparison with the amount of hypertrophy, which was at least 34 per cent for the entire kidney and much greater for the cortical portion.

Although the conclusions from a single case must necessarily be guarded, the results are in agreement with those of Gudden and Peruzzi, who found no evidence of numerical increase in the renal corpuscles after nephrectomy in the newborn rabbit. Whatever may be the factor or factors which determine the number of renal corpuscles and tubules formed in the nephrogenic blastema, it appears that this phase of the developmental process is not influenced by the functional changes resulting from unilateral nephrectomy.

This recalls Roux's doctrine that in the earlier stages of development the organism is essentially self-differentiating, whereas in the later stages the functional and environmental factors participate more extensively in determining the amount of growth.

As to the numerical increase of renal corpuscles in cases of congenital unilateral renal aplasia, it is possible that (as suggested by Peruzzi and others) the conditions are essentially different from those in postnatal renal defects. Possibly the single congenitally enlarged kidney may, through some developmental anomaly, include the embryonic elements normally distributed between the two kidneys. This would not represent a true compensatory hypertrophy at all, but rather a congenital fusion of the two kidneys. A similar explanation might apply to cases of congenitally asymmetric kidneys, with one side apparently atrophic and the other enlarged.

SUMMARY

1. The right kidney was removed in 16 albino rats at ages of 6 to 27 days. After various intervals of 7 to 101 days the rats were killed and the remaining left kidney was found to be 34 to 134 per cent (average 89 per cent) above normal weight.

2. There was some indication of a progressive increase in the amount of hypertrophy in the remaining kidney up to about 6 weeks after nephrectomy, with a decrease later. This conclusion is very uncertain, however, in view of the great individual variability and the correspondingly large probable error.

3. These results, together with those of Arataki, indicate that the amount of compensatory renal hypertrophy is much greater when the nephrectomy is performed in young rats (first month) than in adolescent or mature animals.

4. The rate of compensatory renal hypertrophy also appears more rapid in the younger rats, which usually attain the maximum degree of hypertrophy within 6 or 8 weeks; while in adult rats the maximum is not reached until about 4 months (Moise and Smith).

5. Serial sections of the kidneys in our series reveal no evidence of a preliminary stage of pseudohypertrophy, due to renal congestion, etc., which has been claimed by various investigators. The occurrence of such pseudohypertrophy earlier in the first week, however, is not excluded. Our sections show normal structure (with a few minor exceptions), and no marked congestion, edema, or other abnormality which might account for the renal enlargement.

6. Volumetric analysis of the serial sections throughout one pair of kidneys in our series indicates that the enlargement involves chiefly the cortex, but to some extent also the medulla.

7. Total counts of the renal corpuscles by Kittelson's method in this pair of kidneys indicate no significant increase in the normal number, even when the unilateral nephrectomy had been performed at the age of 7 days, while the process of new formation of corpuscles is still active.

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THE ENAMEL PRISMS AND THE INTERPRISMATIC SUBSTANCE¹

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TEN FIGURES

The dental enamel, because of its peculiarly high inorganic component, is a very difficult tissue to deal with histologically. Until recent years, the methods most commonly employed in studying its minute structure were the preparation of ground and polished sections, isolation of its elements by chemical or mechanical means, and the observation of the frequently meager remains left after decalcification of young incompletely formed enamel. None of these methods was satisfactory by itself and even a combination of all methods gave but an inadequate picture of the finer details of structure of completely formed hard enamel. In ground, polished sections refractive phenomena have undoubtedly been responsible for much confusion and controversy. Isolation by chemical means requires the use of reagents which are insufficiently selective for the elements it is necessary to dissolve in order to free the morphological units of structure, the prisms. Isolation by crushing, chipping, or other mechanical means has but a limited application. Observations made on incompletely formed enamel obviously should not be accepted as conclusive evidence as to the structure of adult enamel without additional proof.

In view of the difficulties involved, it is not surprising that many details of the structure of enamel are still controverted,

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in spite of the fact that many able investigators have attacked the problems.

In recent years an additional mode of attack has been devised, in which the enamel is embedded in celloidin either before or during a process of decalcification, the extremely scanty organic remains of the enamel being thereby retained in their normal positional relation to one another. Such methods have been described by Sudduth ('87) (whose method is usually erroneously ascribed to Fleischmann, '08), Bödecker ('05, '11 a), Malleson ('24), and others. As ordinarily employed, these methods involve carrying the process of decalcification to completion. Under such conditions, only the most meager organic elements are retained, and the resulting preparations have but a limited use in determining the finer structural features. If, on the other hand, decalcification is stopped at a much earlier stage in the process, all the morphological elements of the enamel are retained in their normal relation to one another, yet the tissue can easily be cut into thin serial sections, and these can be stained as desired. This procedure is possible because the enamel, during the progress of decalcification, goes through a stage in which none of its morphological elements is destroyed, even though sufficient inorganic salts have been removed to allow the tissue to be cut readily.

The writer has obtained preparations of this sort by modifications of the methods of Bödecker and Malleson. It is necessary in either case, since dentine decalcifies at a very much slower rate than the enamel, to use pieces of enamel free from dentine, or at least to leave only the thinnest possible layer of dentine adherent. Aside from this precaution, the only modification necessary is a shortening of the period of decalcification to about one-quarter (or less) of that described.

Such sections have many advantages. By decalcification the errors of observation due to refractive phenomena are largely eliminated. Serial sections make it possible to reconstruct the shape of the prisms and their relation to one

another. The fact that all morphological elements are present makes it possible to apply stains which are differential for the component parts and to observe the relation of these elements to each other.

Another method which gave some information was the direct microscopical observation of ground sections during the progress of decalcification. This method, used by many of the early histologists, has considerable worth and should be employed more often than it is nowadays. It involves only the simple procedure of running dilute acids under a cover-slip supported on fine cover-props over a thin ground section of enamel. The acid can be continuously changed by employing a piece of blotting-paper at the opposite side of the cover-slip from that at which the acid is supplied. One per cent nitric, hydrochloric, or sulphuric acids or 5 per cent chromic, acetic, citric, or lactic acids decalcify slowly enough so that all the steps in the process of decalcification can be observed without gas being formed sufficiently rapidly to obscure the picture. The process may be stopped at any stage desired by supplying water instead of acid, and, if sufficient care be used, the section can be stained and mounted in balsam without disturbing the position of the remains.

With the exception of a few preparations of dog's enamel, all the enamel used in this work was obtained from sound adult human teeth, fixed in 10 per cent formalin. The bits of enamel, after decalcification, embedded in their celloidin matrix, were embedded in paraffin and sectioned serially. Sections 4, 6, 8, and 10 μ were cut, the majority being 8 μ . A variety of staining methods was employed, including Delafield's haematoxylin and eosin, Heidenhain's iron haematoxylin and fuchsin, Weigert's iron haematoxylin, fuchsin alone, gentian violet, methylene blue, Disse's stain, and Mallory's connective-tissue stain. The best results were obtained with Mallory's connective-tissue stain and with haematoxylin and eosin combinations, since with these the prisms and interprismatic substance are differentially stained.

Several more or less closely related problems of the structure of enamel, all of which have been the subject of considerable controversy in the past, will be discussed from the standpoint of information gained chiefly from preparations of partially decalcified enamel.

The first problem involves the question of the shape of the enamel prisms. Formerly regarded and still often described as of a somewhat irregular hexagonal prismatic form, they have been described by Smreker ('03, '05) as columns, channeled or grooved longitudinally on one or more sides and convex on one side. Von Ebner ('06) described blade-like alar processes projecting from their sides in much the same manner as do the processes of tendon cells. Smreker's contentions have been attacked by Walkhoff ('04) and Jasswain ('24). Both of these investigators ascribed Smreker's findings to deceptive refractive phenomena, due to obliquity of the plane of section or oblique illumination. J. L. Williams ('23), without ascribing any reasons, likewise denied the presence of either hexagonal or grooved prisms and claimed, as also did Jasswain, and Beckwith and A. Williams ('26), that the cylinder is the typical form of the enamel prism. Von Ebner's alar processes have been denied directly or by inference by Walkhoff, Jasswain, J. L. Williams, Beckwith and A. Williams, Hopewell-Smith ('27), and others.

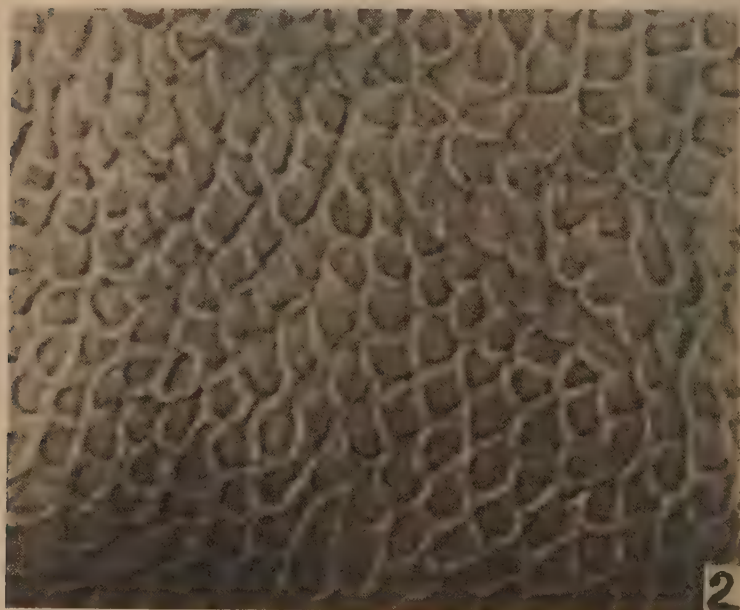
All of the investigators mentioned in the preceding paragraph based their opinions as to the shape of the enamel prisms on study of ground sections of very young decalcified enamel. Ground sections, as mentioned above, on account of their thickness and the inorganic salts in them, are so subject to confusing refractive phenomena that it is unsafe to base definite conclusions on them. Stained sections of partly decalcified adult enamel, however, can safely be relied upon for the accurate determination of the shape of the prisms. For this purpose, sections cut in a plane perpendicular to the long axis of the prisms are most useful.

Study of such sections confirms in all details the observations of Smreker and von Ebner with respect to the shape of

the prisms (figs. 1 to 6). The characteristic shape of the prism is that of a column modified from a hexagonal prism by the concave longitudinal channeling of one, two, or even three of its faces, the remaining faces together forming a single convex, rounded surface comprising one-half or more of the total surface. The prisms of a given group are arranged in fairly regular rows, their convex surfaces all facing in the same direction—toward the dentine. The concave surfaces of prisms of one row fit over the convexities of the prisms of the next row.

The prisms have, as Smreker noted, two main types of arrangement. In the first arrangement the prisms of one row alternate with those of the adjacent rows in such a fashion that the middle of the convex surface of one prism lies opposite the boundary between two prisms of the next row (fig. 4 and most of figs. 1, 2, 3, and 6). In the second arrangement the prisms of adjacent rows lie over one another so that the middle of the convex surface of one prism lies opposite the middle of a prism of the next row (fig. 5 and some areas of figs. 1 and 3).

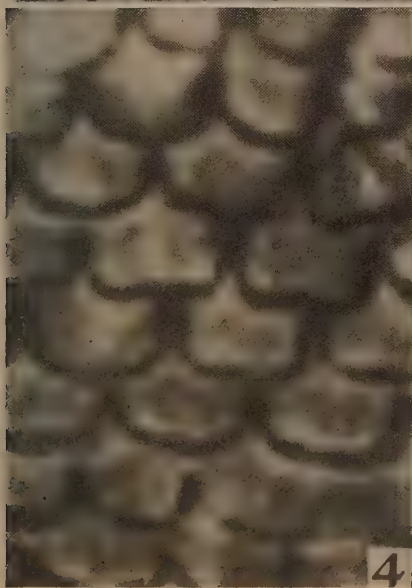
The alar processes of von Ebner are extremely common. They are formed by those portions of the prisms which are included between two concave surfaces or between a concave and a convex surface, hence there are at least two, usually three, places on each prism where it is possible for an alar process to be located. Ordinarily, only the projection bounded by two concave faces achieves sufficient height to be entitled to the name of alar process, though occasionally high ones occur between the concave and convex surfaces. In prisms arranged according to Smreker's first type (fig. 4) each process arises opposite the middle of the convex face, whence it extends a greater or less distance between two adjacent prisms of the next row. In the second type of arrangement of the prisms (fig. 5) two alar processes are ordinarily formed, one at either junction of the usually single concave surface with the convex surface. The alar processes vary in height, from those which scarcely project beyond the contour of the prism,



All figures are from photomicrographs.

Figs. 1 and 2 Sections of partially decalcified adult human enamel cut in a plane perpendicular to the long axis of the prisms and stained with Mallory's connective-tissue stain. $\times 1920$.

Figs. 3 to 6 Sections of partially decalcified adult human enamel cut in a plane perpendicular to the long axis of the prisms and stained with Delafield's haematoxylin and eosin. Figure 3, $\times 1000$. Figures 4 to 6, $\times 3000$, enlarged from a negative $\times 1000$.



Figures 3 to 5

to a height greater than the diameter of the body of the prism itself. In the latter case the process extends into the second row from the one to which its parent prism belongs.

The variation in the height of the alar processes occurs not only from prism to prism, but also along their course on individual prisms. In serial sections they can be traced, in individual prisms, as far as the prism can be identified, now lower, now higher. A wax reconstruction of the interprismatic substance belonging to a group of twelve prisms shows that these changes in height produce an irregularly sinuous edge of the process. Although characteristically unbranched, a few unusually high processes were found which divided into two near their ends.

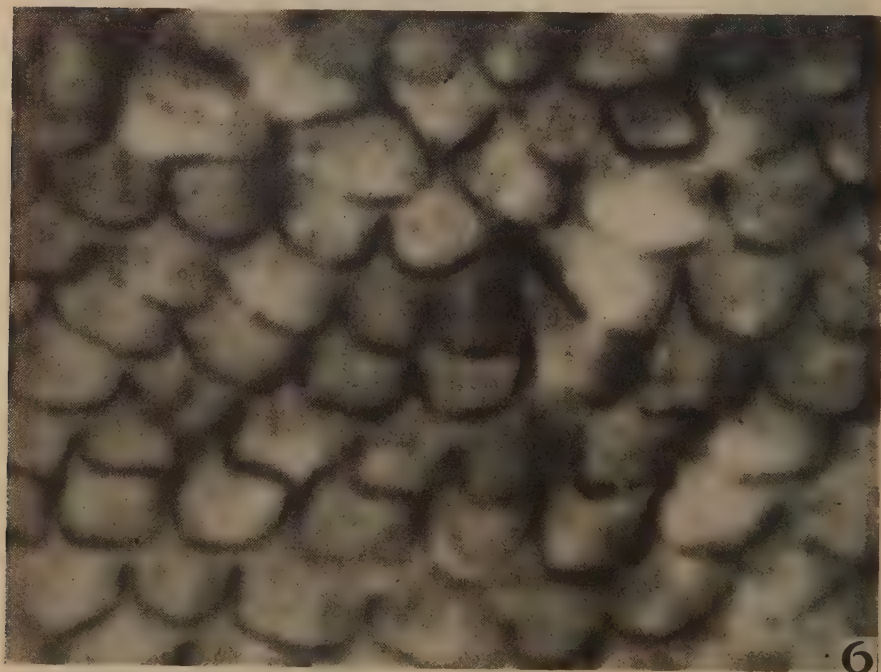


Figure 6

That the concavities and convexities of the prisms and the alar processes can in no way be ascribed to obliquity of the plane of section or to optical phenomena can readily be shown by tracing individual prisms through serial sections. Many prisms were followed in this way (with the aid of enlarged photomicrographs), some of them through as many as twenty-seven sections, each 8μ thick. This procedure and the wax reconstruction of the interprismatic substance (seven sections, each 8μ thick, of twelve prisms) enable one to determine with certainty the shape of the prisms. The individual prisms have approximately the same shape in cross-section, except for variations in the height of the alar processes, over long stretches. Two prisms which had readily identifiable cross-sectional shapes retained these shapes so closely that enlarged outline drawings of them from any two of twenty-seven serial sections could be almost superimposed.

The second problem involves two more or less closely inter-related questions concerning the nature and relation of the structural elements of the enamel. The first question is whether there is an interprismatic substance. The second is whether there is a prism sheath, either with or without an interprismatic substance. The controversy has been prolonged and complex and has resulted in a great diversity of views. Without going into details of the arguments presented, suffice it to say that all of these views can be grouped under three² major beliefs. These are:

1. That the enamel consists of enamel prisms or rods more or less separated from one another by an interprismatic substance (von Ebner, '22; Smreker, '05).

2. That the enamel consists of the prisms alone, each prism being differentiated into an axial core or body and a peripheral layer, the cortex or prism sheath (Walkhoff, '24; Baumgartner, '11; Fischer, '11).

² The old belief that the enamel consists solely of undifferentiated prisms, since it does not appear to be held by any recent writers on the subject, is not included.

3. That the prisms, each surrounded by a prism sheath, are more or less separated from one another by an interprismatic substance (Bödecker, '09; Studnička, '17; Malleson, '24).

As mentioned above, most of the previous work on the minute structure of enamel has been done on ground and polished sections, either etched or not, or on sections of completely decalcified enamel. On account of the marked refractive phenomena present in ground sections, the microscopical examination of such sections allows the observer to interpret the structure in any one of the three ways given in the preceding paragraph, depending on the focal plane employed in making the observation. As the plane of focus is changed, a dark peripheral zone may be caused to appear and disappear in many of the prisms. This might readily be interpreted as a cortical region of the prism differing in nature from the axial region, which remains lighter. Again, both in ground sections and in sections of completely decalcified enamel, the alar processes have probably been responsible for the belief in the presence of an interprismatic substance and a prism sheath existing concomitantly. In ground sections these narrow processes are insinuated between the wider bodies of the prisms and have undoubtedly been mistaken for a special interprismatic substance, the true interprismatic substance being interpreted as a prism sheath—a fact already commented on by von Ebner ('22) and Smreker ('05). Malleson's ('24) description of double lines of organic remains following complete decalcification, interpreted by him as evidence of the presence of prism sheaths separated by interprismatic substance, is, too, probably based on the arrangement of these organic remains in the region of alar processes.

Stained sections of partially decalcified enamel in large measure obviate these difficulties, since the confusing refractive phenomena are almost entirely eliminated and because the prismatic substance and the interprismatic substance can be stained differentially. In haematoxylin and

eosin preparations (figs. 3 to 6) the prisms retain the eosin, while the interprismatic substance takes a strong haematoxylin stain. When Mallory's connective-tissue stain is used (figs. 1 and 2), the prisms are stained with fuchsin, the interprismatic substance with aniline blue. Such differential reaction to double-staining by either of the methods just cited leaves no doubt that there is an interprismatic substance which differs in chemical nature from the substance of the prisms.

In addition to a chemical difference between the substance of the prisms and the interprismatic substance, there is also a marked structural difference. The interprismatic substance is always homogeneous. In sections stained with haematoxylin the staining is so intense that it is difficult to make out the structure, but aniline blue (figs. 1 and 2) and methylene blue (figs. 8 and 9) leave the interprismatic substance clear and transparent, as it also appears in unstained preparations. The prisms, on the other hand, show a coarse longitudinal fibrillation. In cross-sections of the prisms the fibrils appear as dots which have a diameter of about 0.5μ (fig. 6 shows them best). In sections parallel to the long axis of the prisms there is a marked, rather irregular, longitudinal striation (figs. 8 and 9). The matrix in which these fibrils run has a translucent, cloudy appearance. Discrete granules cannot be discerned, even under very high magnification.

There is no sign of a prism sheath in any of my sections of partially decalcified enamel, however prepared. In doubly stained sections there is always a clean-cut line of separation between the acidophile substance of the prisms and the basophile interprismatic substance, and the cortical zone of the prism does not differ from the axial portion.

Walkhoff's conception, according to which the enamel is composed of prisms alone, each prism being differentiated into an axial portion, or body, and a cortical portion, the prism sheath, would require, besides the interfaces between the prism sheaths and the bodies of the prisms, other interfaces between contiguous prisms. If he has regarded the

interprismatic substance as his prism sheaths, and if his view were correct, we should expect sections to show the interprismatic substance bisected throughout by a line indicating the interfaces between his prism sheaths. No such line appears in any of my preparations (figs. 1 and 2).

The only places in any of my preparations of partially decalcified enamel which could possibly be interpreted as

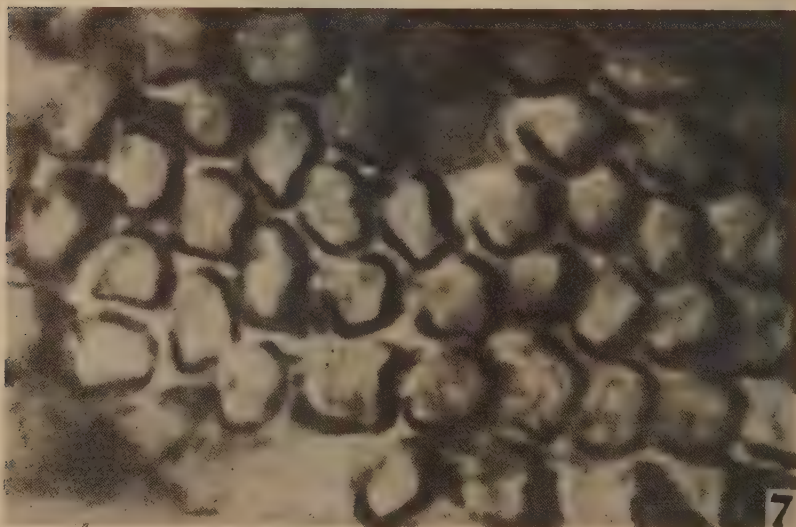
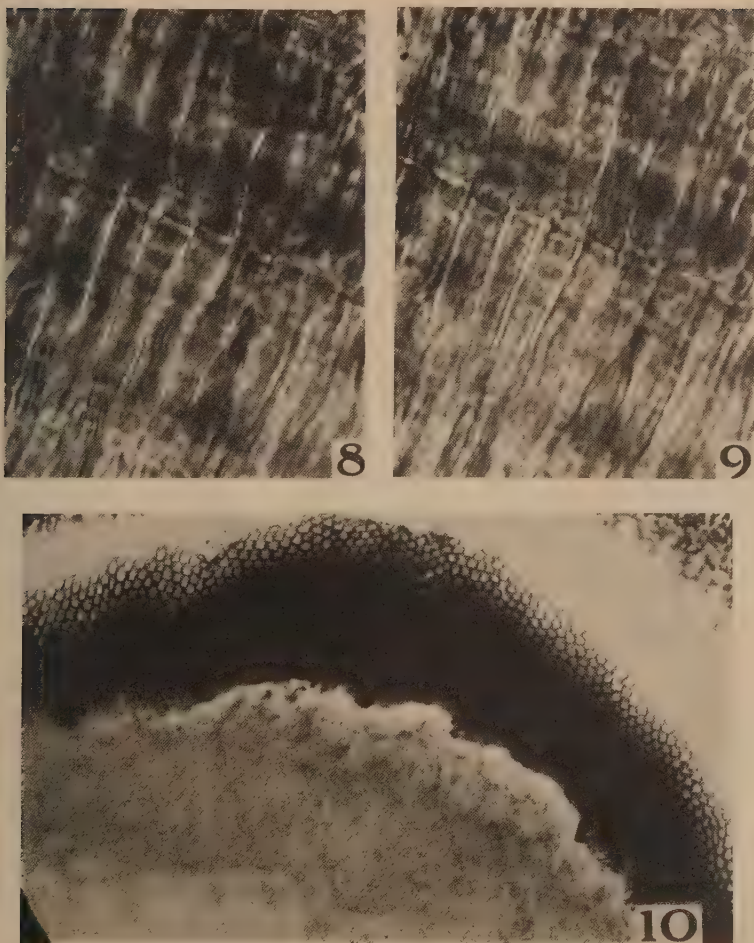


Fig. 7 Ground section of enamel from young dog. Impregnated, before grinding, by Bielschowsky method. $\times 1710$.

possessing prism sheaths are those in which alar processes are inserted between prisms. In serial sections it can readily be shown that these processes always are connected with the bodies of enamel prisms, hence cannot represent a special interprismatic substance lying between prism sheaths.

With regard to the distribution of the interprismatic substance and its relation to the prisms, my observations afford almost complete confirmation of the description given by Smreker ('05). Smreker used ground sections of very young enamel in which the interprismatic substance was either



Figs. 8 and 9 The same area of a preparation made by partially decalcifying a ground section of adult human enamel (cut in a plane parallel to the long axis of the prisms) under a cover-slip, then staining with methylene blue. Figure 8 shows a higher focal plane than does figure 9. Note change in appearance of margins of prisms occurring with slight change of focus. Intraprismatic fibrils well shown. $\times 1000$.

Fig. 10 Celloidin section (25μ) passing through the uncalcified developing enamel near the cervix of an erupting premolar (dog). The Tomes' processes of the ameloblasts, pulled from the interprismatic substance, leave the latter in the form of a honeycomb. Haematoxylin and eosin. $\times 255$.

impregnated with silver nitrate, stained with fuchsin by Rupperecht's method, or unstained. He found that the interprismatic substance is not equally applied to all surfaces of the prisms, being limited almost exclusively to the convex surfaces, hence, in sections across the prisms, forming a series of arches, to which he applied the name of 'arcades.' He distinguished two types of arcades: one in which the arches belonging to the prisms of one row are confluent at their sides (between adjacent prisms)—continuous arcades—and one in which the arches of adjacent prisms do not conjoin—discontinuous arcades.

All of these appearances described by Smreker for very young enamel are corroborated for adult human enamel by my preparations. Figures 1 to 6 show the continuous type of arcades throughout. Figure 7, of dog's enamel, shows the discontinuous type, which is rare in human enamel.

In all of these figures regions can be seen in which the interprismatic substance, as it is indented between adjacent prisms of a given row, does not meet the interprismatic substance of the next row. In such situations there is a direct continuity of the prismatic substance from prism to prism. These structures, which may be named interprismatic bridges, are especially prominent in the lower parts of figures 4 and 5 and in the lower left corner of figure 6. They are of variable length. Some have been traced through as many as six sections, each 8μ thick, but many appear in only one such section. The thickness, too, varies from less than 1μ to more than 3μ .

Smreker believed that the interprismatic substance is present in the interprismatic bridges, though not in a stainable condition. He maintained that he could demonstrate it between the silver-impregnated, separate arches of his discontinuous arcades in ground sections of newly formed enamel by treating the sections with dilute hydrochloric acid. My experience with adult human enamel has been quite the reverse of this. Neither by the employment of Smreker's method nor in any of my stained preparations of partially decalcified enamel has any trace of interprismatic substance been found

in the interprismatic bridges. The material of the interprismatic bridges has the same structural appearance and the same staining reaction as the material of the bodies of the prisms and continues, without discernible interruption, from prism to prism. In other words, there is an actual discontinuity of the interprismatic substance in places where the interprismatic bridges occur.

How this discontinuity arises I have not been able to discover. The prismatic and interprismatic substances are different chemically and structurally from the beginning on, so that they can readily be distinguished from each other in stained sections. The interprismatic substance is first deposited in a continuous mass surrounding the Tomes' processes of the ameloblasts. The substance between adjacent Tomes' processes is of approximately uniform thickness and the spaces occupied by the Tomes' processes and by the enamel prisms formed in and by them are tubes which are typically hexagonal in cross-section, so that the interprismatic substance, taken as a whole, has very much the appearance of a honeycomb (fig. 10). If the enamel prisms and the interprismatic substance were calcified without alteration of this early structure, the enamel would consist of hexagonal prisms separated from one another by an interprismatic substance of uniform thickness. After calcification, however, the prisms are in the form of columns, channeled lengthwise on the side away from the dentine and convex on the side toward the dentine; the interprismatic substance, at least in adult enamel, is restricted to the convex surfaces of the prisms, and there are frequent breaks in the continuity of the interprismatic substance through which adjacent prisms become conjoined. To get from one condition to the other we must assume the presence, during organization of the enamel, of mechanical forces operating to cause the alteration in shape of the prisms and also the transfer of the interprismatic substance from the side of the prism which becomes channeled to the side which becomes convex. Obviously, these changes must occur while the enamel is still plastic; but how they occur must

remain for the present undetermined. Smreker's hypothesis is ingenious, but unconvincing, in view of the fact that it requires the retention of the interprismatic substance, uniformly thick, but regionally physically altered, completely isolating the individual prisms. This condition is certainly not realized in adult enamel.

The belief is popular that the interprismatic substance remains in an uncalcified state. Opinions range from the conception of Bödecker ('09), Halmar-Jungnar ('05, cited by von Ebner, '22) and Adloff ('14), who believed in a fluid interprismatic substance, to that of von Ebner ('22), who believed it completely calcified in fully formed, normal enamel. Between these extreme views there are also many intergrades wherein the interprismatic substance is regarded as more or less incompletely calcified. The conception of an uncalcified or only relatively calcified interprismatic substance is grounded on the ability to impregnate or stain this substance under certain conditions, notably those of extreme youth and imperfect formation. Von Ebner and others have shown, and the writer can reiterate, that the interprismatic substance of well-formed adult enamel can neither be stained nor impregnated without previous partial decalcification. When the process of decalcification of ground sections is observed under high magnification, minute bubbles of gas can be seen to arise from the interprismatic substance as well as from the substance of the prisms. These pieces of evidence, taken together, signify at least a high degree of calcification for the normal interprismatic substance.

That regions exist in adult enamel where the interprismatic substance is imperfectly calcified or incompletely formed is not to be doubted, but the writer is convinced that these regions are developmental defects resulting in faulty structure of the adult enamel. The only too common attempts to ascribe to these structures the rôle of pathways of metabolic interchange should be curbed until far stronger evidence than any yet advanced has been accumulated. Among the structures involving incomplete or imperfect calcification are the

enamel spindles, enamel tufts, and enamel lamellae, on which structures work now in progress will be reported in a later paper.

The intraprismatic fibrils described on page 249 are in all probability derived from the fibrils of like appearance which are found in the Tomes' processes of the ameloblasts. In completely decalcified enamel these fibrils comprise the only formed organic remains, the interprismatic substance and the surrounding portions of the prisms being either completely dissolved or leaving only amorphous remains. Bödecker ('11 b) has interpreted these fibrillar remains as interprismatic—an error easy to make if one deals with decalcified enamel only. Examination of a series of preparations covering a range from partially decalcified to completely decalcified enamel, however, readily convinces one of the fact that the fibrillar remains following complete decalcification are intraprismatic rather than interprismatic elements.

In none of my preparations of partially decalcified enamel has there been any sign of spaces which could in any way be interpreted as homologous with the enamel tubules of marsupial enamel or as the enamel canals described by Williams ('23).

SUMMARY AND CONCLUSIONS

Dental enamel can be partially decalcified, leaving remains in which all morphological elements are retained in normal positional relation. Examination of stained serial sections of these remains gives new evidence to aid in the settlement of several much controverted problems concerning the shape, structure, and interrelation of the structural elements of the enamel. The observations reported in this paper were, for the most part, made on normal, completely formed human enamel. They lead to the following conclusions:

1. The conclusions of von Ebner and Smreker with respect to the shape and arrangement of the enamel prisms are correct in all details. The typical shape of the prism is that of a column, modified from the form of a hexagonal prism by the lengthwise channeling of one, two, or three of its faces and

the alteration of the remaining faces to form usually a single convex face. Each convex surface lies on the side of the prism which is directed toward the dentine. The prisms are typically equipped with one, two, or three alar processes which resemble the processes of tendon cells in form. The prisms of a given group are arranged in rows. The prisms of one row either alternate with those of the next row or lie over them.

2. The enamel consists of the enamel prisms, more or less completely separated from one another by an interprismatic substance. The partially decalcified interprismatic substance is basophile, homogeneous, and transparent. The partially decalcified prisms are acidophile, longitudinally fibrillated, the fibrils being embedded in a cloudy, translucent matrix. The interprismatic substance differs from the substance of the prisms chemically, structurally, and histogenetically.

3. There is no prism sheath.

4. The interprismatic substance, in adult enamel, tends to be limited to the convex surfaces of the prisms where it forms the arches described by Smreker and, by the fusion or non-fusion of individual arches of a row of prisms, the continuous or discontinuous arcades. Failure of the interprismatic substance of one row of prisms to join with that of the next row is a common feature. In such places the prismatic substance is continuous from prism to prism forming structures named the interprismatic bridges.

5. The interprismatic substance of well-formed adult human enamel is calcified to a high degree. There is as yet insufficient evidence for regarding this substance as the pathway of metabolic interchange in the enamel.

6. The fibrillar remains following complete decalcification are intraprismatic rather than interprismatic elements.

7. No enamel tubules or enamel canals could be found.

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THE MORPHOLOGY OF THE FROG'S KIDNEY

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SIX FIGURES

INTRODUCTION

The frog's kidney has been an object of both anatomical and physiological study since the work of Nussbaum ('86). More recently, however, the amphibian kidney has been studied in the living condition chiefly from the standpoint of a renewed interest in renal function. Such studies have necessitated a very accurate knowledge of the distribution of the tubules and their relation to the dorsal and ventral renal surfaces. A review of the papers dealing with the morphology of the amphibian renal tubule shows clearly, however, that complete anatomical data have not been available. Fairly adequate descriptions and figures of models (some reconstructed in wax and some by serial sections) are given in the literature (Nussbaum, '86, and Beissner, '99), but none of these indicates the exact relationship of tubule to kidney. Nussbaum depicts very accurately an isolated unit tubule of the frog's kidney closely comparable to that shown in figure 5 of this paper. Beissner also depicts unit tubules in *Rana fusca* and *Rana esculenta* which, while diagrammatic, indicate the essential relation of the segments of the tubule to each other. Holmes, in his recent "Biology of the Frog," gives diagrams showing the general relation of renal tubules, glomeruli, and collecting ducts to each other.

Within the past few years, Edwards and Marshall ('24) and Edwards ('26) have pointed out that only distal convolutions appear on the ventral surface and only proximal

convolutions on the dorsal surface of the mesonephros in the living frogs which they studied. This finding, if constant, is important from the standpoint of physiological considerations. However, in the previous morphological investigations (Nussbaum and Beissner) this relationship is not adequately touched upon, so that no complete anatomical demonstration of such a distribution of tubules in reference to the surfaces of the kidney is available. Therefore, it is not because of essentially inadequate data concerning the anatomy of the frog's kidney and its topographical relations that this anatomical reexamination has been undertaken, but rather to establish definitely by dissection and observation this one fundamental point, namely, the exact relation of the convolutions of the renal tubules of the frog to the surfaces of the kidney.

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MATERIAL AND METHODS

The kidneys of *Rana pipiens* and *Rana catesbiana* were used in this study. The method of maceration employed by Traut ('23) in his work on the human kidney was adopted with modifications. In applying this method to the frog's kidney it was first necessary to determine a concentration of hydrochloric acid just strong enough to soften the strongly resistant basement membrane between the tubules and the glomeruli. After many trials with various strengths of acid, it was found that a solution of hydrochloric acid from 40 to 42 per cent is most satisfactory for *Rana catesbiana* and 37 per cent for *Rana pipiens*, since the latter has a kidney possessing a markedly less resistant basement membrane.

Before putting the kidney in acid, forced injections of Prussian-blue reagents (iron ammonium citrate and potassium ferrocyanide) were made through the descending aorta just below the coeliac axis, after the iliac arteries and the renal portal veins had been ligated. When the Prussian blue was subsequently precipitated by acid, it made the glomeruli stand out and thus facilitated dissection of a given tubule, since the glomeruli could then be more easily seen and

used as starting-points. The Prussian-blue reagents were prepared as follows:

A. Iron-ammonium citrate,	5 grams
Distilled water,	500 cc.
B. Potassium ferrocyanide,	5 grams
Distilled water,	500 cc.

The two solutions were mixed and filtered as needed. After the injection the kidneys were put into the acid solution. This solution served a twofold purpose: the Prussian blue was precipitated from the injected solution and at the same time the tissue macerated. When the tissue was allowed to remain in the acid from twelve to fourteen hours, maceration progressed uniformly throughout all parts of the kidney without causing any disintegration of the tubules. If allowed to remain for fifteen to twenty-four hours longer, the tissue became very soft and disintegrated very readily so that dissection was impossible. After the requisite number of hours in the acid, the kidneys were removed and rinsed carefully six or eight times in distilled water and then kept for several days in distilled water to which a small amount of thymol had been added. Preserved in this way, the tissues swell and separate to such an extent that isolation of the individual tubules is fairly easy. All dissections were made with the tissue under water and with the aid of a Greenough binocular microscope. For teasing out the tubules, glass needles of the required size were prepared by drawing out glass rods.

In the study of the collecting tubules the maceration process was not entirely satisfactory, owing to the large amount of very tenacious connective tissue surrounding them. This made the dissection difficult, especially in the neighborhood of the ureter where the connective-tissue sheet is very firm. Because of this difficulty, the ureter was injected in a further series with a thin solution of celloidin which hardened in situ and formed a cast of the tube and its tributaries. These specimens were macerated until the connective tissue and tubules had completely disintegrated, so that casts of the collecting ducts could be obtained for study.

RESULTS

a. Topographical relations

A cross-section of the frog's kidney is approximately hemi-circular. The ventral surface is quite flat and the dorsal surface is curved to adapt itself to the bony curvature of the spinal column. This topography, with minor variations, is shown by observation and dissection of transected blocks taken at various points along the longitudinal axis of the kidney. The cross-section shown in figure 1 is typical. In this figure the inferior vena cava, the adrenal gland, the distal convolutions and glomeruli are indicated on the ventral surface. On the dorsal surface only the proximal convolutions and occasionally a branch of the collecting tubules are observed. The ureter and renal portal vein are situated dorsolaterally.

By regarding this transaction in its entirety one is immediately impressed by the segregation into zones of the various parts of the unit tubule. The proximal convolutions are quite uniformly restricted to the dorsal third of the kidney, indicated as a crescentic zone—*Zone Z* in figure 1. One finds also in this area practically the entire system of collecting tubules, with the exception of the terminal branches, which extend ventrally to join the distal convolutions. Similarly, the distal convolutions are chiefly found in a ventral zone designated as *Zone X*, although their proximal and distal ends extend into the intermediate or central zone. This latter, *Zone Y*, consists of the glomeruli all grouped together in a striking manner in the form of a crescent, as first mentioned by Nussbaum. In this zone one also sees the transition of proximal to distal convolutions by means of the tubular necks and the formation of the collecting ducts from the distal convolutions.

b. The tubule

From its origin in the glomerulus, the tubule extends dorsally from Bowman's capsule by a narrow short neck into

the straight, first portion of the proximal convolution. The convolutions begin near the dorsal surface of the kidney, but extend subsequently into the deeper portion of the dorsal zone. The pattern of the convolutions in individual tubules

EXPLANATION OF FIGURES

All figures are camera-lucida drawings.

ABBREVIATIONS

<i>G.</i> , glomerulus	<i>C.D.</i> , collecting duct
<i>N.</i> , neck of proximal convoluted tubule	<i>U.</i> , ureter
<i>P.C.</i> , proximal convolution	<i>E.P.V.</i> , renal portal vein
<i>T.N.</i> , tubular neck or narrow portion of tubule connecting the proximal and distal convolutions	<i>V.C.</i> , vena cava
<i>D.C.</i> , distal convolution	<i>V.S.</i> , ventral surface
	<i>D.S.</i> , dorsal surface
	<i>C.T.</i> , connective tissue

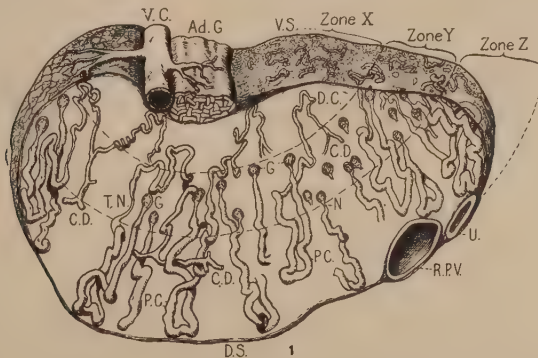


Fig. 1 Diagram of cross-section of a kidney, cut midway in its long axis, showing, as a result of an in-situ dissection, the relation and distribution of glomeruli and tubules as follows: ventral zone (*Zone X*), distal convolutions; middle zone (*Zone Y*), glomeruli, the neck and upper portions of the proximal convolutions, the tubular neck and parts of the distal convolutions and collecting ducts; dorsal zone (*Zone Z*), proximal convolutions and collecting ducts. With two-thirds reduction of diagrams, size as printed is $\times 20$.

is extremely variable. About midway between the dorsal and ventral surfaces, the proximal convolution is connected with the distal portion of the tubule by a markedly narrowed, straight part (the tubular neck, *T.N.* of figs. 1 to 5). This narrow straight segment, including the segments of proximal

and distal convolutions which it unites, occupies the central glomerular zone, *Zone Y*. It is always found in close proximity to the glomerulus from which the tubule has its origin. In *Rana catesbiana* it is attached to the glomerular wall by so tenacious a band of connective tissue that normal maceration is insufficient to detach it (fig. 4, *C.T.*).

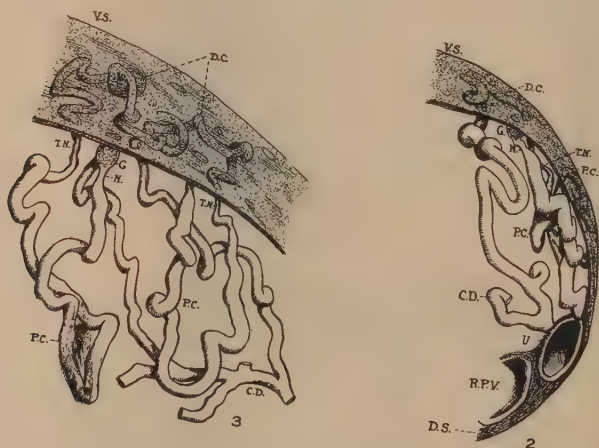


Fig. 2 Lateral view of a kidney, showing arrangement of marginal tubules on the ventral surface near the portal veins. Figures 2 to 5, inclusive, represent characteristic unit tubules as they appear in a hemisection of the kidney at practically any point along its long axis: figure 2 at the lateral edge, figure 3 near the lateral border, figure 4 in the midportion beneath the adrenal body, and figure 5 at the medial edge. These figures, although exhibiting minor variations in the arrangement of the tubules in conformity with the kidney contour, illustrate the uniformity of structure of the unit tubule. $\times 40$.

Fig. 3 Showing the arrangement of the tubules on the ventral surface near the lateral border. $\times 120$.

The distal convolutions, seen in the ventral third of the kidney and at the surface, are more closely coiled, have a much smaller diameter, and are of shorter length than the proximal convolutions. They also lie near the glomerulus from which the tubule takes its origin. The general relation and location of these convolutions are shown in figures 1 to 5.

To determine the constancy of this arrangement, dissections were made of transected blocks taken at various points

along the long axis of the kidney, and also laterally and medially. Essentially, the structure of the unit tubule remained unchanged, regardless of the locality from which the tubule was taken. Minor variations in tubular arrangement are necessary to conform with the kidney contour, as seen in figures 1, 2, and 3. The pattern of the unit tubules in situ is some-



Fig. 4 Showing the arrangement of tubules in the central part of the kidney as they lie in the vertical dorsoventral plane beneath the adrenal gland. In this region the distal convolutions do not extend parallel to the ventral surface for any considerable distance, being for the most part at right angles to it. $\times 120$.

Fig. 5 Showing the arrangement of several distal convolutions on the ventral marginal surface from the middle of the kidney. The two tubules shown end in a common collecting trunk. The distal convolutions appear to be less intimately associated with the renal capsule in this portion of the kidney than in the lateral portions, as is illustrated in figure 2. $\times 40$.

what radiate from ventral to dorsal surface. The proximal convolutions which are larger and longer than the distal ones are thus nicely fitted in the greater space provided by the curvature of the dorsal surface. Since the area of the ventral surface is much less than the dorsal, it is obvious that a radiate arrangement would obtain where the proximal convolutions constitute more than one-half of the kidney and the distal convolutions much less than one-half.

This uniform scheme is only occasionally interrupted by abnormalities in tubular and glomerular development. Many variations in size and length are observed, some tubules being extremely short and others longer, larger, and more coiled than the average tubule. The commonest anomaly is found in the neighborhood of the ureter where tubules having only a proximal convolution empty directly into the ureter. Some



Fig. 6 Reproduction of a mass injection of the ureter and the collecting-duct system. This illustrates the very irregular arches, branches, and anastomoses of the collecting-duct system. Seven orders of collecting ducts can be counted in some places in this figure. The total number of orders within the kidney could not be accurately determined, however, and the terminal collecting-duct branches, as represented in this figure, are not to be taken as an indication of their junction with distal convolutions. $\times 30$.

of these are quite long and narrow, while others are strikingly short and very thick. One example of a double proximal convolution was noted. Its proportions were extreme and the glomerular end was forked into two branches which joined two quite large glomeruli. The other parts of this unit were entirely normal. A similar occurrence is depicted by Beissner ('99), plate IX, figure 11, for *Rana esculenta*. Typical examples of the average tubules are seen in figures 4 and 5.

c. Collecting ducts

Since the collecting ducts are an integral part of the tubular unit, their relation to the tubule and ureter was studied in

some detail. Numerous large trunks are given off from the ureter and extend horizontally toward the middorsal region. From these large trunks smaller and smaller branches extend until a maximum of seven can be made out by the method employed. A general idea of the relationship is obtainable from figure 6. However, the indicated terminals of many of the ducts, farthest removed from the large trunks into which they empty, are not to be taken as an indication that they join the distal convolutions at that point. Under the conditions of injection and dissection of the ramifications of the collecting-duct tree, it was not possible to make out in many instances the junction of the collecting duct and its distal convolution. It is most probable that there are many more than seven orders in the collecting-duct system.

COMMENT

The method of maceration and dissection is a most satisfactory way to determine accurately the minute internal topography of such an organ as the frog's kidney. It is of value, moreover, because it can be so accurately controlled that it is possible to separate tubular units in the exact form in which they lie within the kidney capsule. Their consistence in a good preparation is such that the normal curves and convolutions are sustained even after they are separated by dissection. A simple experiment was carried out to establish this point. The ureter of an isolated kidney, after it had been clamped off midway between the cephalic and caudal poles of the kidney, was injected with a preparation of very dilute India ink under moderate pressure. Since in the frog the collecting ducts enter the ureter at numerous points along the lateral edge of the kidney, the superior collecting ducts are occluded, whereas the inferior ones still remain in continuity with the ureter below the point of occlusion. This allows a massive injection of the tubules and glomeruli up to the point at which the ureter is obstructed. Beyond this rather sharply defined zone occasional tubules become injected, because a few of the terminal branches of the collect-

ing ducts extend cephalad beyond the level from which they join the ureter. When such a preparation is cleared according to the Spalteholz technique, individually injected tubules can be observed in situ. In no way are such isolated tubules found to differ from the units previously described.

This study was undertaken as an anatomical contribution, of interest particularly, because the frog's kidney has been universally adopted for functional studies in vivo. In the experiments of several observers there has been some difference of opinion as to the relation of the distal and proximal convolutions to the ventral and dorsal surfaces, and there is no really accurate description of these relationships in the literature. The structural pattern described in this paper has been suggested by Edwards and Marshall ('24) and Edwards ('26), who noted the difference in reactions of dyes in the tubules on the dorsal and ventral surface. Their observations could be made only along the lateral edge of the kidney where it is thinnest and where the network of superficial vessels found medial to the adrenal body is lacking. Moreover, in the living kidney microscopic study is facilitated by dorsoventral compression of the organ. However, in the interpretation of results, it must be remembered, as shown in figure 2, that a few segments of proximal convolutions can normally be seen along this extreme lateral edge on the ventral surface, and that, moreover, compression will tend to increase the number of proximal tubules in the field. It seems important to avoid this extreme lateral portion in physiological observations upon distal convolutions on the ventral surface. With this one possibility of error in mind, the anatomical dissection substantiates the observation that only distal convolutions are seen on the ventral surface and proximal ones on the dorsal surface.

SUMMARY

1. By means of dissections of kidneys of *Rana pipiens* and *Rana catesbiana*, using the maceration technique of Traut, the relation of proximal and distal convolutions to the dorsal and ventral surfaces has been established.

2. The kidney is quite definitely divided into three zones: a ventral zone, composed of distal convolutions; a middle zone, composed of glomeruli and connecting portions of the tubules, and a dorsal zone, composed of proximal convolutions and collecting ducts.

3. The unit tubule is for all practical purposes invariable in its structure. Its distribution varies only in its adaptation to the kidney capsule.

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VESTIGIAL AND PROVISIONAL URINIFEROUS TUBULES ABSENT IN THE METANEPHROS OF BIRDS

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TWO FIGURES

In 1919, Kampmeier discovered vestiges of uriniferous tubules in the vicinity of the primitive pelvis of the kidneys in a 30-mm. human embryo. One of these vestigial structures was attached to the epithelium of the renal pelvis.

Subsequent studies ('23-'24) carried out by him on an extensive series of human embryos and fetuses disclosed that such vestiges were not anomalies, but occurred constantly. Because of their nature and position, he interpreted them as a generation of uriniferous tubules belonging to the first order of collecting ducts. He further discovered that the second and third generations of uriniferous tubules, though joined to the corresponding collecting ducts and perfectly formed and functioning early perhaps, later degenerated. The malpighian corpuscles of these generations were characterized by a notably large size before they and their tubules disappeared, many of them by cystic degeneration.

On the basis of these observations, Kampmeier suggested that not only the pronephros and mesonephros are retrogressive organs, but that the metanephros also is in small part vestigial.

The present study on the developing metanephros of birds was undertaken to compare it with that of man and to determine: 1) whether in birds there is a generation of uriniferous tubules corresponding to the vestigial one in man; 2) if so,

whether it is functional or retrogressive, and, 3) whether or not any succeeding generation of uriniferous tubules is transitory and subsequently undergoes reduction.

This investigation was carried out on serially sectioned chick embryos ranging from four days of incubation to forty-eight hours after hatching. Before indicating the findings, the literature on the development of the metanephros of birds—which is not extensive—may be briefly considered.

Bornhaupt, in 1867, observed that, at the end of the fifth and sixth days of incubation, the anlage of the metanephric blastema appeared as an area of darkly staining cells dorsal to the posterior end of the mesonephros. The primary ureter bud grew into this mass, the primary collecting ducts branched off as buds from the ureter, while the secondary collecting ducts arose from the metanephric blastema in the vicinity of the primary ones and later fused with them—a view which has since been shown to be incorrect.

In 1897, Sedgwick carried out a cursory study of the metanephros of the chick until the eighth day of incubation. He noted that the collecting ducts developed as a series of buds from the ureter, but he presented no observations on the formation of the uriniferous tubules.

According to Schreiner ('02), the primitive ureteric bud springs as a diverticulum from the wolffian duct in embryos of six days; and some twelve hours later, the anlagen of the first order of collecting ducts arise from it. They early (seven days) give origin to the second order of ducts by branching. The anlagen of the uriniferous tubules appear at nine days as vesicles of cylindrical epithelium in close proximity to the apices or ampullar ends of the collecting ducts. At approximately ten days, these vesicles have elongated, have formed S-shaped tubules, and have developed the Bowman's capsule. Junction with the collecting ducts is not established until twenty-four hours later.

Huber's studies ('17) have shown that the uriniferous tubules of the metanephros in birds closely resemble those of mammals not only in regard to the malpighian bodies, but

in the possession of proximal and distal convoluted tubules and ascending and descending limbs of Henle's loop.

Rienhoff ('22) followed the development of the uriniferous tubules of the chick in tissue cultures. He observed that in small transplanted particles of the metanephros the tubules acquire their predestined form regardless of the foreign environment. He found that the convoluted tubule arises as a result of the differential growth of its component cells and actually noted the union of the convoluted and collecting tubules.

In embryos of seven days examined by the writer, the anlagen of the first generation of collecting ducts had already been laid down by outgrowth from the primary ureter, some of them having branched to form the secondary generation of ducts. At eight days, these had increased to four generations, the mode of branching during their formation being partly dichotomous and partly monopodial. As yet there are no sharply defined anlagen of the uriniferous tubules, but caps of blastema exist, covering the terminal ends or ampullae of the third and fourth order of collecting ducts.

No isolated masses of blastema, like those described by Kampmeier in human embryos, were found centrally or in the vicinity of the primitive ureteral pelvis; nor were anlagen of uriniferous tubules observed in relation to the primary and secondary orders of collecting ducts.

Vesicles of cylindrical epithelium form from the metanephric caps in the chick at nine days, but are still separate from the adjacent collecting ducts. Continuity between these two structures is established in the ten-day stage. At this time the uriniferous tubules have attained their S-shaped condition; subsequently, at twelve, thirteen, and fourteen days, these tubules acquire their definitive form as described by Schreiner and Huber. These earliest tubules were followed through the later stages of chick embryos, but no evidence of their degeneration was detected. Nor were isolated uriniferous tubules or their remains observed near the pelvis or the kidney.

The oldest tubules were found to join the third order of branches of the renal pelvis; none were found in connection with the primary and secondary branches (fig. 1).

The change in staining reaction which begins to take place at twelve and one-half days suggest that the tubules are

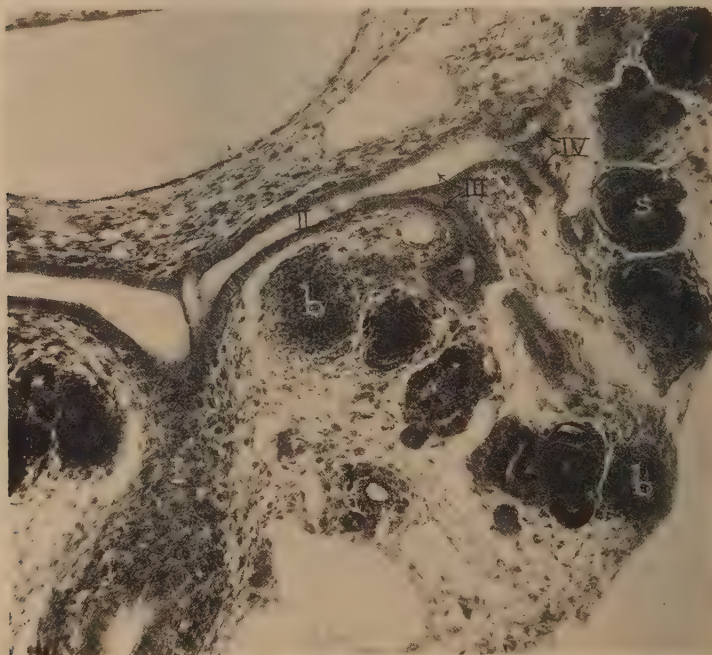


Fig. 1 Photomicrograph of a transverse section of the kidney of a chick embryo of eleven days' incubation. *b*, metanephric blastema; *s*, S-shaped anlagen of uriniferous tubules; *I, II, III, IV*, primary, secondary, tertiary, and fourth generations of collecting ducts.

beginning to function. They acquire an acidophilic reaction which becomes widespread, until, at fourteen days, the entire kidney with the exception of a few newly formed tubules at the periphery reveal this change.

The kidney of the newly hatched chick showed no evidence of any degenerative changes in any of the uriniferous tubules.

The tubules seemed normal throughout the kidney and gave evidence of being actively functional. A notable difference between the avian and the human kidney also obtains with regard to the fate of the earliest orders of collecting ducts.

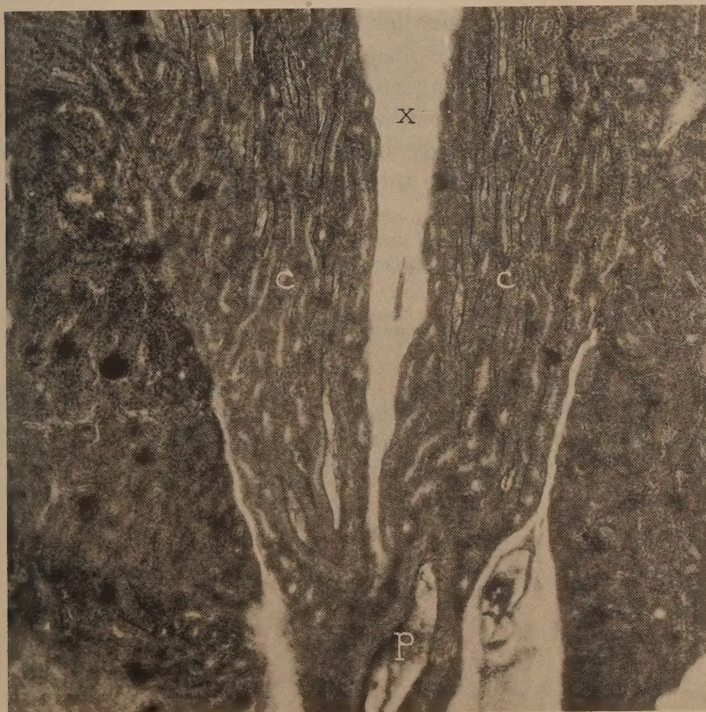


Fig. 2 Photomicrograph of a sagittal section of the kidney of a newly hatched chick (forty-eight hours after hatching). *p*, common papillary or primary collecting duct; *c*, collecting ducts of later orders; *x*, a tear due to shrinkage.

Whereas these in the human become incorporated in the expanding renal pelvis, in the newly hatched chick they were found to have been retained and to open into the renal pelvis by a common papillary duct and aperture, as illustrated in figure 2, which channel must be interpreted as a primary collecting duct.

A comparison of the relative diameters of the malpighian bodies of the central and peripheral areas of the metanephros in various stages of chick embryos reveals no marked disparity between the central and most peripheral malpighian bodies. As indicated in the accompanying table, the peripheral ones are slightly smaller than the centrally located, but no giant glomeruli were observed in the bird, as noted by Kampmeier in the human embryo. It can be seen that the largest malpighian bodies were present in the kidney of the newly hatched chick, there being a gradual increase in size of the glomeruli from the thirteenth day on.

Showing size of peripheral and central glomeruli of metanephros of chick embryos

AGE OF EMBRYO	DIAMETER OF CENTRAL GLOMERULI	DIAMETER OF PERIPHERAL GLOMERULI
	μ	μ
Thirteen days	37.6	32.5
Fourteen days	42.4	39.3
Newly hatched chick (forty-eight hours)	45.5	42.8

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